

*Evaluation of a high throughput nucleic acid extraction method for PCR-based detection of Mycobacterium avium ssp. paratuberculosis in bovine fecal samples*

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Johne's disease (paratuberculosis) is an economically important disease of cattle worldwide. The disease is caused by *Mycobacterium avium* subspecies paratuberculosis (MAP) and manifests as a chronic inflammation of the intestine in ruminants leading to loss of production, culling and mortality. Although, culture is considered the gold standard for the disease diagnosis, PCR is used in several diagnostic laboratories due to its rapid turnaround time, with sensitivity and specificity comparable to fecal culture. The objective of this study was to evaluate a high throughput nucleic acid extraction method for the detection of MAP in bovine fecal samples by PCR. We used the MagMAX™ CORE Nucleic Acid Purification Kit with Mechanical Lysis Module for extraction of DNA from bovine fecal samples. The MagMAX™ CORE Nucleic Acid Purification Kit uses a magnetic separation process for purification of nucleic acid and the Mechanical Lysis Module involves a bead beating step for efficient lysis of bacteria. Initially, the 2018 Johne's disease proficiency test individual and pooled panels, provided by the National Veterinary Services Laboratories (NVSL), were tested using the MagMAX CORE kit extraction protocol followed by PCR amplification using a MAP DNA test kit (Tetracore). Each individual panel consisted of 25 blinded samples and one positive control. The method correctly categorized all individual samples from non-shedding (6), high-shedding (9) and 80% (8 out of 10) of low and moderate-shedding animals. The pooled proficiency test samples consisted of 4 positive and one negative and all were correctly categorized. The new extraction method was further evaluated using 51 known Johne's positive and 6 known negative diagnostic fecal samples previously tested at the Pennsylvania Veterinary Laboratory. Of the 51 positive samples, 8 were culture positive and 43 were PCR positive. The previous DNA extraction method involved isolation of DNA on a BioRobot M48 workstation using the MagAttract DNA M48 Mini Kit (Qiagen). Use of the new extraction method resulted in categorization of all 57 diagnostic specimens correctly except one. The sensitivity and specificity of detection for the new method based on analysis of the diagnostic samples were 98.84% and 100%, respectively. In addition, 9 lab-prepared pooled fecal samples, each containing 4 known negative and 1 known Johne's positive fecal samples, were tested. Of the 9 pooled samples, 8 samples were correctly categorized. The data presented in this study demonstrate an efficient extraction of MAP DNA from bovine fecal samples using MagMAX CORE extraction protocol with mechanical lysis module for sensitive diagnosis of Johne's disease in cattle. The new extraction method is rapid and allows high throughput testing of samples.