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ABSTRACT BELOW

Direct Evaluation of Bovine Fecal Samples for *Mycobacterium avium* subsp. *paratuberculosis* by a Real-Time PCR Commercial Assay

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An evaluation of a new real-time PCR commercial kit (VetAlert™ from Tetracore, Inc.) for the direct detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from bovine stools was conducted. The VetAlert kit utilizes MAP-specific primers and a MAP-specific FAM-labeled probe, in an optimized, pre-made master mix, for sensitive and specific detection of DNA by real-time fluorescent probe hydrolysis. Utilizing a positive control plasmid containing the cloned amplicon, the VetAlert real-time PCR assay was shown to have sensitivity down to 1 gene copy. Concurrently, a novel stool extraction kit for simplified extraction of DNA from stool compared to conventional procedures was also evaluated. The new extraction procedure combines mechanical disruption with chaotropic solid phase extraction. Under current configurations, 18 stool samples were processed and extracted in 1.5 hours and 50 samples took less than 4 hours. Twenty-five fecal samples from the National Veterinary Services Laboratories Johne's Check Test were extracted and evaluated with both the VetAlert test kit and by nested PCR (Collins et al., 1993, Vet Microbiol 36:289). Samples were blinded to the operator, run in duplicate, and, in the case of real-time PCR, evaluated on both the ABI 7700 and Cepheid SmartCycler real-time instruments.

Of the 25 extracted fecal samples, there was 100% correlation between positive and negative results from the VetAlert test and nested PCR. Likewise, results of the real-time VetAlert test were comparable on both the ABI and Cepheid instrument platforms. Real-time PCR and nested PCR were shown to have equally high sensitivity; however, real-time PCR is a very attractive diagnostic test because it can be performed faster than nested PCR, it is less labor intensive, and it is quantitative.

In conclusion, real-time PCR is a sensitive, specific, and rapid diagnostic method which allows for direct evaluation of fecal samples for *M. avium* subsp. *paratuberculosis*.

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