Comparison of Fecal Culture and Direct Fecal Real-time PCR in the Identification of Mycobacterium avium subsp. paratuberculosis in Fecal Specimens

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ABSTRACT
Eight hundred seventy-one fecal specimens from dairy cows were analyzed in a comparative study of methodologies using the Trek® fecal culture and Tetracore® real-time direct fecal PCR diagnostic systems. Of the 109 positive fecal cultures, real-time PCR identified 40 (36.9%). Of the 662 negative fecal cultures, real-time PCR identified 56 (8.3%) as being positive.

INTRODUCTION
Heat-shock proteins are present in all organisms under normal temperatures. Heat-shock proteins (HSP 90, HSP 70, and HSP 60) are induced by cells in response to a variety of stimuli: raised temperature, starvation, oxygen radicals, toxins, and viral and bacterial infections. When an organism is phagocytized by a neutrophil, heat-shock protein production by the initiating organism is significantly increased. Heat-shock protein 60 (HSP 60) is the dominant antigen induced by mycobacterium, Coxiella burnettii, legionella, treponema and borrelia infections (Tizard, 2004). The real-time PRC test (Tetracore®) measures heat-shock protein X (HSP X). The purpose of this paper is to further analyze the sensitivity and positive predictive value of a positive fecal real-time PCR test based upon the Map hsp X protein relative to positive fecal culture Map isolates.

MATERIALS AND METHODS
Study population: The fecal samples were obtained from two dairy herds that participated in The Florida Johne’s Disease Dairy Herd Prevention Program. Animals within the Florida Johne’s Disease Demonstration Project. A total of 871 individual fecal specimens were available for analysis.

Diagnostic tests: The Trek® (fecal culture) and the Tetracore® (real-time PCR) Map Diagnostic Systems were utilized in accordance with their respective manufacturers’ instructions.

Culture confirmation PCR: Samples that show positive acid fast staining were tested using IS900 PCR primers.

RESULTS
Eight hundred seventy-one fecal specimens were analyzed using the Trek® fecal culture and the Tetracore® real-time direct fecal PCR diagnostic systems. Of the 109 positive fecal cultures, real-time PCR identified 40 (Table 1). Of the 662 negative fecal cultures, real-time PCR based upon HSP identified 56 as being positive (Table 1). The false positive percentage was 8.3%.

<table>
<thead>
<tr>
<th>Number of specimens</th>
<th>culture +/</th>
<th>culture +/</th>
<th>culture -/</th>
<th>culture -/</th>
</tr>
</thead>
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<tr>
<td>871</td>
<td>109/</td>
<td>109/</td>
<td>662/</td>
<td>662/</td>
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<tr>
<td></td>
<td>40/</td>
<td>69/</td>
<td>606/</td>
<td>56/</td>
</tr>
</tbody>
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Table 1. Correlation of fecal culture identification of Map with fecal real-time identification of Map.
DISCUSSION
The Tetracore® information brochure contains a reference study in which frozen fecal archived samples from 221 cows were evaluated in a blind trial comparing test results from the *Mycobacterium Paratuberculosis* DNA Kit, Polymerase Chain Reaction test with culture on HEYM (Anon., 2008). The fecal samples included 100 samples from non-infected cows and 121 culture positive samples of varying intensities. All samples (100/100) from the non-infected cows correctly gave a negative result in the test. The kit gave a positive test result in 59/60 cases when samples had an average cfu/tube count of greater than 3. When samples contained less than 3.0 cfu/tube, the kit gave a positive test result in 45/61 cases (Anon., 2008).

The Tetracore® test results from the Florida Johne’s Disease Prevention Dairy Herd Monitoring Program do not support the proficiency data claimed by Tetracore in their brochure of March 19, 2008. In this study, the Tetracore test correctly identified 40 of the 109 culture positive cows. An addition 56 culture-negative fecal specimens were identified as being positive in the Tetracore test system.

The test results published by Tetracore in March 2008 were based upon preselected specimens and the comparisons were made to culture identification achieved with HEYM. Heat-shock protein X is not unique to Map. A variable not addressed by Tetracore in their study was cross-identification of heat-shock protein from other pathogenic mycobacterium such as *Mycobacterium avium*, subspecies avium (Ma), *M. bovis*, genomic variants between Map and Ma.

CONCLUSIONS
If herd management decisions are based upon Tetracore data, additional comparative herd studies need to be done to determine the extent to which the *Mycobacterium Paratuberculosis* DNA Kit, Polymerase Chain Reaction Tests correctly identifies Map in a given fecal specimen.

REFERENCES