The Prevalence of Possible *Mycobacterium Avium* Subspecies *Avium* in Fecal Sample from Dairy Cows

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**ABSTRACT**

A comparative study of fecal culture, hspX Map real-time PCR and nested 1311-based PCR tests was undertaken to determine the incidence of positive fecal test results using IS1311-base nest PCR primers relative to fecal culture and real-time PCR using hspX.

Three hundred sixty-eight fecal samples from the Florida Johne’s Disease Dairy Herd Demonstration Project had been analyzed using fecal culture, real-time PCR and nested PCR for the detection of *Mycobacterium avium* subsp. *paratuberculosis* (Map). Forty-one fecal specimens tested positive by the direct fecal nested Map PCR test (FecaMap®). In 34 of the cases, the corresponding real time PCR test for Map was also positive. Mycobacterium isolates were achieved by fecal culture in 21 of the 41 cases. In 20 of the 21 cases of culture recovery of a mycobacterium, IS900-based primers confirmed Map. In the remaining case, fecal culture demonstrated case heavy growth and the corresponding hspX real time PCR were both positive. In the remaining 6 direct nested PCR tests, no evidence of mycobacterium growth was present. Assuming fecal culture to be 100% sensitive, the project herd false-positive incidence using IS1311 based nested primers would be approximately 1.1%.

**INTRODUCTION**

The Linda strain of *Mycobacterium avium* subspecies *paratuberculosis* (Map) that established the IS900 insertion sequences as the definitive marker of mycobacterium that cause Johne’s diseases was deemed a centralist stain representative of the group (Harris and Barletta, 2001). It has been subsequently argued that the IS900 insertion sequence is a vertical cut through a horizontal evolutionary process emanating from *Mycobacterium avium* subspecies *avium* in which exist polymorphic variants of these two species that can cause Johne’s disease in herbivores and omnivores (Frothingham, 1999; Turenne et al., 2007). In horses, pigs, and dogs, Ma and *Mycobacterium avium* complex (Mac) are the causative agents of Johne’s disease (Turenne et al., 2007). The FecaMap® and LactoMap (Infectious Diseases Incorporated, Bellevue, NE) direct and nest PCR test primers were developed using the IS1311 insertion sequence in order to effectively span the potential spectrum of pathogenic mycobacterium as well as to provide tests applicable to avian species and zoo animals not identifiable using commercial Map ELISA tests.

Despite demonstrated pathogenicity in horses, pigs, dogs, and selected zoo animals, there is a tendency to consider Ma as an environmental mycobacterium rather than a pathogenic mycobacterium. The argument can be advanced that use of PCR tests based upon the IS1311 insertion sequence would only result in a significant number of false-positive results.

The purpose of this study was to analyze to what degree using a IS1311-based PCR test would positive results be identified that were not substantiated by fecal culture or real-time PCR using hspX.

**MATERIALS AND METHODS**

**Study population:** Three hundred sixty-eight dairy cows within the Florida Johne’s Disease Dairy Herd Demonstration Project constituted the study population. Selection of a cow was predicated upon prior independent analysis of its feces using the FecaMap® nested Map PCR test.
**Fecal cultures**: Fecal cultures were done at Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Purdue University using the Trek® Map Culture System in accordance with the manufacturer’s instructions.

**Real-time Map PCR tests**: Real-time Map PCR tests were done at Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Purdue University using Tetracore® Map Extraction and DNA test kit in accordance with the manufacturer’s instructions.

**Direct fecal nested Map PCR tests**: Direct fecal nested Map PCR tests were done at University of Florida College of Veterinary Medicine using the FecaMap® system in accordance with the manufacturer’s instructions. The FecaMap® direct primers recognize a 242 base pair sequence of Map IS1311 and its nested primers overlap and span a 104 base pair region within the insertion sequence.

**RESULTS**

Three hundred sixty-eight fecal samples from the Florida Johne’s Disease Dairy Demonstration Project had been analyzed. Out of 368 fecal samples, Ma/Map was identified by the IS1311 primer of the FecaMap® test (1.1%).

Forty-one fecal specimens tested positive by the nested Map PCR test. In 34 of the cases, the corresponding real time PCR test for Map was also positive (Table 1). *Mycobacterium* isolates were achieved by fecal culture in 21 of the 41 cases. In 20 of the 21 cases of culture recovery of a mycobacterium, IS900-based primers confirmed Map.

In the remaining case, fecal culture demonstrated case heavy growth and the corresponding hspX real time PCR was positive. The animal was culled before the need to retest was identified. Six fecal samples identified by the IS1311 nested PCR were not substantiated by either fecal culture or real-time PCR using hspX.

**DISCUSSION**

Despite covering only 6-8 copies, the direct IS1311 direct and nested primers were demonstrated to be more sensitive in identifying Map contained within USDA’s Laboratory Certification Tests.

The overall incidence of a positive IS1311-based nested Map PCR test was 1.1%. A positive nested reaction had an 85% probability of identifying Map; however the remaining 14% of positive nested PCR tests were not confirmed by their corresponding fecal real-time PCR and culture tests. More importantly, the nest Map PCR identified a non-IS900 mycobacterium whose test profile was that of being a heavy shedder in the Trek® culture system and of testing positive in the Tetracore® PCR system. These observations coupled with early culling makes it, more likely than not that this animal had a significant mycobacterium infection. Culture data demonstrating heavy shedding would have been disregarded had diagnostic confirmation been done using IS900 primers. To what degree other non-IS900 potentially pathogenic mycobacteria have been dismissed as being environmental contaminants is unknown. In this small series, the presence of a non-IS900 presumably pathogenic mycobacterium was calculated to be 2.4%.

**Table 1.** Analysis of dairy cows in the Florida Johne’s Disease Prevention Dairy Herd Demonstration Project for prevalence of Map/Ma DNA in fecal samples as determined by the FecaMap® direct nest fecal Map PCR test.

<table>
<thead>
<tr>
<th># of fecal specimens</th>
<th># culture +/ nested -</th>
<th># RT PCR +/- nested +</th>
<th># non-Map + cultures/ RT &amp; nested +</th>
</tr>
</thead>
<tbody>
<tr>
<td>368</td>
<td>20/41</td>
<td>34/41</td>
<td>1/1</td>
</tr>
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CONCLUSION
IS1311-based nested Ma/Map primers done on fecal 368 fecal samples tested positive for 1.1% of the specimens, had an 85% positive correlation with the probable presence of Map, but had a 14% presumed false positive rate.

If IS1311 PCR primers are used to confirm mycobacterium culture isolates derived from fecal or milk cultures, the IS1311 primers appear to identify non-IS900, pathogenic mycobacterium.

REFERENCES

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