Effect of season and lactation stage on the diagnostic sensitivity of direct real-time PCR assay for detection of fecal shedding of *Mycobacterium avium* subsp *paratuberculosis*

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Introduction

Mycobacterium avium subsp *paratuberculosis* (MAP) is the causative organism of Johne's disease. Dairy Farmers of Canada lists this production-limiting disease as one of the top two animal health priorities for the Canadian dairy industry. Infection of newborn calves from ingestion of MAP-infected feces, colostrum, or milk is considered the main route of transmission and a major concern in a herd. Current diagnostics rely on identification of the bacterium in feces via culture and molecular tests or identification of MAP antibodies in milk or serum via enzyme-linked immunosorbent assays (ELISA). However, these tests are inadequate to meet industry needs for herd biosecurity and environmental-transmission control of MAP, especially when MAP-infected cows are in the subclinical stages of the disease. Milk ELISAs have poor predictive values due to imperfect sensitivity and specificity, especially when used in herds with a low prevalence of MAP-infected cows. Although culture of fecal samples for MAP is currently the gold standard diagnostic test for identification of MAP-infected cows, the long incubation times, costs, and intermittent shedding of MAP in feces hinder its use as an efficient screening tool. The goal of this study was to assess how shedding patterns of MAP in feces vary with lactation stage and season, as determined via direct real-time polymerase chain reaction (PCR) assay.

Materials and Methods

For this study, 51 confirmed MAP-positive cows from seven Atlantic Canadian dairy farms were purposively selected from data collected for a companion project. These cows were confirmed MAP-positive by fecal broth culture (TREK ESP), acid-fast stain (AFS), and real-time PCR assay (Tetracore). Samples of feces were collected monthly from July 2010 through December 2011, depending on the first month of farm participation and for as long as the cow remained in the herd. Cows chosen for the study were primarily Holstein and had an average parity of two (range, 1 to >7). Farms had primarily free-stall housing, and ranged in size from 83-490 cows per herd. All fecal samples collected were subsequently frozen at –112°F (–80°C) until processed. Direct real-time PCR methods were performed by the use of the IS900 insert in accordance with the instructions for the TetracoreVet Alert Johne's Real-Time PCR kit. Statistical analysis was performed via a mixed logistic model with STATA11 and Minitab 16, and values of $P < 0.05$ were considered significant.

Results

From the 51 confirmed MAP-positive cows, 390 fecal samples were collected and 78% of those tested positive for MAP via the direct real-time PCR assay. Days-in-milk (DIM), season, and potential confounding effects for farm were analyzed in the model. Farm and DIM were not significant predictors for a fecal sample to yield positive MAP results on direct real-time PCR assay. However, season was significantly ($P = 0.019$) associated with the probability that a fecal sample from a MAP-positive cow would test positive via direct real-time PCR assay. The mean probability of the test being positive in the summer and fall seasons was 92% of the mean probability of the test being positive in the winter and spring seasons.

Significance

Detection of MAP in fecal samples via a direct real-time PCR assay provides results quickly, is less costly than culture, and avoids the use of decontamination steps that could inadvertently decrease already low numbers of MAP in some samples. Results of this study indicate that for known positive cows, there was a high sensitivity of MAP detection with a direct real-time PCR assay; therefore, the use of a direct real-time PCR assay to test fecal samples for MAP is a viable option as a screening tool for Johne's disease control programs at the herd level. Furthermore, the significant difference in PCR test sensitivity between the summer/fall and winter/spring seasons leads to questions regarding the impact of herd management and housing on MAP fecal shedding. Further study into this is on-going.