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Reliability of environmental sampling to quantify *Mycobacterium avium* subspecies *paratuberculosis* on California free-stall dairies

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ABSTRACT

The reliability of environmental sampling to quantify *Mycobacterium avium* ssp. *paratuberculosis* (MAP) based on collector and time was evaluated. Fecal slurry samples were collected using a standardized protocol simultaneously by 2 collectors of different experience levels. Samples were collected from 30 cow pens on 4 dairies every other day on 3 occasions while cow movements between pens were minimal. The 4 study herds had moderate MAP seroprevalence and were housed in free-stall dairies in central California. Results of testing the environmental samples for MAP using PCR and culture were strongly correlated. The reliability of environmental sampling simultaneously by different collectors as estimated by the intraclass correlation coefficient was excellent (81%) for PCR and good (67%) for culture and may justify comparison of quantitative results of samples collected by different investigators. The reliability of environmental sampling over a 5-d period was good (67 and 64% for PCR and culture results, respectively), which justifies the utility of environmental sampling to identify pens with a high MAP bioburden between routine cow pen changes on a dairy. Environmental sampling of free-stall pens using the standardized sampling protocol yielded comparable PCR and culture results across collectors with different experience levels and at different times within a 5-d period.

Key words: reliability, environmental sampling, *Mycobacterium avium* ssp. *paratuberculosis*, quantitative real-time PCR

INTRODUCTION

Environmental samples, such as fecal slurry from dairy pens, can be used to detect *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**). In the United States, environmental samples are used for classification of MAP herd status for the Voluntary Bovine Johne's Disease Control Program. The Voluntary Bovine Johne's Disease Control Program allows MAP-negative environmental samples as an alternative to MAP antibody-negative samples from 30 individual cows for entry into the test-negative component of the program (USDA, 2006). Environmental samples were also used in the National Animal Health and Monitoring System Dairy 2002 study (USDA, 2005) and Dairy 2007 study (USDA, 2008a) to estimate the national herd-level prevalence. The sensitivity of the nonstandardized environmental sampling protocol used in the Dairy 2002 study to detect herds with at least 1 serum ELISA-positive result was estimated to be 76% (Lombard et al., 2006).

Recently, a standardized sampling protocol to detect MAP on a dairy using a single investigator collecting environmental samples from common locations was found to have comparable herd sensitivity to ELISA testing and pooled fecal culture (Berghaus et al., 2006). In addition, findings from a simulation study comparing 5 testing strategies indicated that MAP culture of 6 to 10 environmental samples was the most cost-effective method for initial classification of MAP herd status in moderate- and high-prevalence herds and of comparable cost and sensitivity with ELISA in low-prevalence herds in the Midwest of the United States (Tavornpanich et al., 2008).

The use of quantitative tests to estimate MAP concentrations in environmental samples collected from individual pens, rather than common locations such as the wastewater lagoon and return alleyway, may be used to rank pens on a dairy according to the MAP bioburden. Subsequently, cows in pens with the highest environmental MAP bioburden may be preferentially tested, provided MAP concentrations in pen environmental samples can be estimated before routine moving of cows. Typically, cows are routinely moved between

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pens on a California dairy every 1 to 2 wk to adjust for changes in milk production and feed rations; hence, moving may result in a substantial change in pen environmental MAP concentrations. If quantitative real-time PCR (**qrt-PCR**) results could be correlated with culture results on Herrold's egg yolk medium (**HEYM**), which is often considered the most appropriate reference test for MAP in live animals (Collins et al., 2006), then qrt-PCR might be used as a rapid test to rank pens by MAP bioburden.

The utility of environmental samples beyond classifying a herd as MAP infected or not depends in part on the reliability of sample collection and processing, both of which can be characterized in a sampling protocol. A reliable environmental sampling protocol yields samples with less variability attributable to the collection procedure, including collector and time of sampling, than variability attributable to true differences between samples; hence, it yields samples that are comparable and representative of MAP fecal shedding by the cow populations sampled. Other sources of variability in environmental samples are related to cow factors and laboratory testing.

The use of a standardized sampling protocol (Berghaus et al., 2006) should minimize variability through implementation of a specific sequenced procedure that increases the consistency and uniformity with which samples are collected, processed, handled, and shipped. However, the reliability of environmental sampling when performed by different collectors, such as from several herds in a region or from the same herd over time, has not been studied to our knowledge. In addition, the ideal time for collection of environmental samples representative of the current pen population, and changes in MAP concentration over time in a pen, may be investigated through testing environmental samples collected repeatedly over time and when movement of cows between pens is absent or minimal.

The objective of this longitudinal study was to estimate the reliability of environmental sampling to quantify MAP concentrations in fecal slurry samples from 4 free-stall California dairies based on collector and time while adjusting for pen and dairy sampled. Samples were collected using a standardized sampling protocol and were evaluated by qrt-PCR and culture on HEYM.

MATERIALS AND METHODS

Study Herds

Four central California dairies were enrolled in the study. Both dairy 1 (1,676 Holsteins) and dairy 2 (3,577 Jerseys) had routine fecal cultures and serum ELISA testing for MAP at dry-off. Dairies 1 and 2 had a moderate seroprevalence of MAP of 3.5 and 4.5%, respectively, based on ELISA testing at dry-off. In addition, environmental samples were collected and cultured quarterly for MAP as part of the National Johne's Disease Demonstration Herd Project (USDA, 2002). More than 80% of fecal slurry samples collected from these herds in 2006 and 2007 were MAP positive on culture. Dairies 3 and 4 (1,326 and 1,166 Holsteins, respectively) were candidate herds from earlier Johne's disease studies and had whole-herd ELISA testing performed in 2004 and 2006, with similar MAP seroprevalence of 3.8 and 4.6%, respectively, based on the whole-herd ELISA tests.

Lactating cows on all 4 dairies were housed in free-stall pens that were flushed with wastewater from the storage lagoon. Cows on dairy 1 were moved between pens once every 2 wk based on changes in milk production, whereas cows were moved out of the fresh-cow pen every 1 to 2 wk, depending on pen density. On dairy 2, cows were moved at the end of each week, and in dairies 3 and 4 cows were moved at the beginning of the week. On each dairy, environmental samples were collected for the purpose of this study from all the pens housing the entire adult cow herd, specifically from 8, 11, 7, and 4 pens from dairies 1, 2, 3, and 4, respectively. Cow numbers ranged from 105 to 418 cows per pen, with a median of 226, 255, 195, and 301 cows on dairies 1, 2, 3, and 4, respectively.

Study Period

Environmental samples were collected every other day on 3 different occasions from dairies 1 and 2 between November 16 and November 21, 2006, and from dairies 3 and 4 between May 30 and June 3, 2007. Sampling dates were selected to be immediately after routine moving of cows between pens. Herd managers were also asked to minimize moving cows between pens sampled during the study period, with the exception of new hospital entries and discharges, which the managers were requested to document and report to collectors.

Collection of Environmental Samples

Sample collection began 1 to 2 d after scraping the concrete pathways connecting the 2 sides of each free-stall pen (crossover alleyways) to avoid sampling fecal slurry unrepresentative of cows housed in the sampled pens and took place in the mornings at approximately the same time. Two veterinarians (RA and SA) simultaneously collected environmental samples from all 4 dairies. Collector 1 (RA) regularly collected environmental samples quarterly from California herds