

Evaluation of Environmental Sampling and Culture to Determine *Mycobacterium avium* subspecies *paratuberculosis* Distribution and Herd Infection Status on US Dairy Operations

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ABSTRACT

The objectives of this study were to determine the distribution of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the environment and assess the relationship between the culture status of MAP in the farm environment and herd infection status. The National Animal Health Monitoring System's Dairy 2002 study surveyed dairy operations in 21 states. One component of the study involved collection and culturing of environmental samples for MAP from areas on farms where manure accumulated from a majority of a herd's cows. Operations were selected for inclusion based on perceived risk factors for MAP infection identified in a previously administered questionnaire. Individual animal and environmental samples were collected and used to determine the efficiency of environmental sampling for determination of herd infection status. Individual animal fecal, serum, and milk samples were used to classify herds as infected or not infected based on the presence of at least one test-positive animal in the herd. A total of 483 environmental samples (approximately 5 per farm) were collected, and 218 (45.1%) were culture-positive for MAP. A similar percentage of environmental cultures collected from all designated areas were positive [parlor exits (52.3%), floors of holding pens (49.1%), common alleyways (48.8%), lagoons (47.4%), manure spreaders (42.3%), and manure pits (41.5%)]. Of the 98 operations tested with the environmental sample culture, 97 had individual serum ELISA results, 60 had individual fecal culture results, and 34 had individual milk ELISA results. Sixty-nine of the 98 operations (70.4%) had at least one environmental sample that was culture-positive. Of

the 50 herds classified as infected by fecal culture, 38 (76.0%) were identified by environmental culture. Two of the 10 operations classified as not infected based on individual animal fecal culture were environmental culture-positive. Of the 80 operations classified as infected based on serum ELISA-positive results, 61 (76.3%) were identified as environmental-positive, whereas 20 of the 28 (71.4%) operations identified as infected based on milk ELISA were detected by environmental sampling. Environmental sample culturing is less costly than individual animal sampling, does not require animal restraint, and identified more than 70% of infected operations. Environmental sampling is another diagnostic tool that veterinarians and dairy producers can use to determine herd infection status for MAP.

Key words: environmental sampling, paratuberculosis, dairy

INTRODUCTION

Johne's disease (**JD**), or paratuberculosis, is a chronic, progressive disease of ruminants caused by the effects of *Mycobacterium avium* subspecies *paratuberculosis* (**MAP**) infection on the gastrointestinal tract. It is transmitted primarily through a fecal-oral route, especially from manure and environmental contamination by infected adult cattle, and young cattle are most susceptible (Sweeney, 1996). The disease is manifested in adult cattle, leading to economic losses associated with decreased milk production, early culling, and decreased carcass weight. Control of this disease has become a focus of programs in several countries (Sockett, 1996; Kennedy and Benedictus, 2001).

In recent years, many JD control programs have focused on the use of pooled fecal cultures to screen herds for MAP infection status. For small herds with a low prevalence and large herds with a high prevalence, pools of 5 to 10 samples/pool have proved to be valid and cost-

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effective (Kalis et al., 2000; Wells et al., 2002; van Schaik et al., 2003; Wells et al., 2003; Tavornpanich et al., 2004). However, this method has not been appropriately sensitive for medium to large dairy herds with a low prevalence of MAP infection (Wells et al., 2002). The pooled fecal culture method requires individual cow sampling and can vary in sensitivity based on the stage of disease and the shedding level of animals sampled.

The presence of MAP in the dairy environment is believed to be a risk factor for the spread of MAP through a herd. *Mycobacterium avium* subspecies *paratuberculosis* has been shown to survive in manure slurry long-term at temperatures approaching freezing (Jorgensen, 1977). A more recent study has shown increased duration of MAP survival in shaded soil and pasture (Whittington et al., 2004). Lack of shade was shown to decrease survival, presumably because of infrared wavelengths and temperature flux. This evidence suggests that MAP survives for long periods of time in common dairy farm environments. However, the distribution of MAP in the dairy environment is not well documented. Samples collected from areas on dairy operations where manure accumulates from a majority of adult animals are commonly referred to as environmental samples. A study conducted in Minnesota by Raizman et al. (2004) found the most common areas to obtain culture-positive samples were cow alleyways and manure storage areas. Environmental sampling detected 78% of known infected herds identified by previous testing and 95% of herds in which the concurrent pooled fecal culture was positive. Berghaus et al. (2006) performed a similar study on 23 California dairies and found that lagoon water was the most likely to yield a positive culture and that environmental sampling identified 65 to 74% of infected operations identified by previous or concurrent individual animal testing. Further understanding of the relationship between MAP distribution, the environmental culture method, and other standard screening tests would be useful in further developing the environmental culture as a herd-level screening method.

The objectives of this study were 1) to determine the distribution of MAP in the environment and herd characteristics associated with culture-positive environmental samples on US dairy farms and 2) to assess the relationship between culture status of MAP in the farm environment and herd infection status as determined by individual cow fecal culture, serum ELISA, and milk ELISA.

MATERIALS AND METHODS

Herd Selection

States included in the National Animal Health Monitoring System Dairy 2002 study were selected to represent at least 70% of the animal and producer populations

in the United States. Data from the USDA, National Agricultural Statistics Service (NASS), were used to determine the major US dairy states based on animal populations. Operations were selected from a stratified random sample of the NASS list frame to participate in phase I of the study, whereas those operations that had completed phase I (which consisted of a questionnaire), had 30 or more dairy cows, and were willing to continue to participate were included in phase II. Final selection for phase I included operations from 21 states from 4 regions of the United States and represented 83.0% of dairy cattle operations and 85.7% of dairy cattle (USDA, 2002). Data collected from phase I included herd size (small = less than 100 cows, medium = 100 to 499, and large = 500 or more), region and state (West = California, Colorado, Idaho, New Mexico, Texas, and Washington; Midwest = Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio, and Wisconsin; Northeast = New York, Pennsylvania, and Vermont; Southeast = Florida, Kentucky, Tennessee, and Virginia), breed, rolling herd average (RHA) milk production, and housing type (multiple, individual, and none). Information on herd characteristics, operation history of JD (including animals with clinical signs, defined as chronic diarrhea and weight loss that did not respond to treatment despite a normal appetite), and previous testing were collected in phase II.

A subset of herds participating in phase II were selected to participate in environmental sampling and within-herd MAP prevalence testing—using fecal culture and serum ELISA—based on the operations' perceived risk of having and transmitting MAP. The operations eligible for phase II were then ranked from high to low risk based on perceived risks factors from their answers from phase I and included 1) number of dairy cows, 2) how soon calves were separated from their dams, 3) whether pooled colostrum was fed, 4) the percentage of dairy cows that had diarrhea for more than 48 h, 5) whether maternity housing was separate from lactating cow housing, 6) how many replacements were brought onto the operation during 2001, and 7) whether the operation required JD testing for purchased cattle. Perceived low- and high-risk herds were identified from each participating state, with herds stratified into below and above median herd size for the state. Between 4 and 6 operations were selected in each participating state. Two operations were chosen from the low-risk group—one above and one below the median herd size for the state. Three operations were chosen from the high-risk group—either 2 above the median herd size and one below the median herd size, or vice versa. From the operations selected, a further subset of herds that participated in DHIA testing was also asked to participate in milk ELISA testing.

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