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Prevalence of Michigan dairy herds infected with *Mycobacterium avium* subspecies *paratuberculosis* as determined by environmental sampling

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ABSTRACT

A cross-sectional, stratified random survey of Michigan dairy herds was conducted to estimate the prevalence of herds infected with *Mycobacterium avium paratuberculosis* (MAP), the causative agent of Johne's disease, in Michigan using targeted environmental sampling. One pooled sample each from the primary manure storage area and a high-traffic common cow area from each herd was collected and cultured for MAP using the ESP® culture system II. A herd was classified as positive if at least one sample was culture positive for MAP. State, agricultural district, and herd size stratum prevalence were calculated. Information on past MAP testing and cattle purchase history was collected, and logistic regression was performed to determine their importance to the MAP status of the herd. One hundred twenty-seven herds were contacted, and 94 agreed to participate in the study. The environment of 38 (40.4%) herds cultured positive for MAP. MAP was found in all herds ($n = 15$) with greater than 200 lactating cows. Herds that had tested for MAP or purchased cattle in the previous 5 years were 4.6 and 3.1 times, respectively, more likely to be infected than herds that had not. MAP continues to be prevalent on Michigan dairy farms, especially those with greater than 200 lactating cows. The environmental sampling protocol used in this study is an economically attractive alternative for monitoring herd level prevalence and the progress of Johne's disease control programs at the state or national level. Implementation of such a program would aid states in monitoring Johne's control program progress, and guide changes over time.

1. Introduction

Johne's disease (JD) is an infectious disease of cattle and other ruminants caused by the bacterium *Mycobacterium avium paratuberculosis* (MAP), resulting in a slowly progressive granulomatous enteritis, weight loss, diarrhea, and eventually death. The NAHMS Dairy 1996 study estimated the prevalence of dairy herds infected with MAP in the US to be 21.6% (USDA, 1997), but other estimates range from 21% to 93%, depending on region and testing method used to classify infected herds (Collins et al., 1994; Obasanjo et al., 1997; Thorne and Hardin, 1997; Johnson-Ifearulundu and Kaneene, 1998; Johnson-Ifearulundu et al., 1999; Adaska and Anderson, 2003; Hirst et al., 2004). Johne's disease costs the US dairy industry an estimated $200–250 million annually due to primarily reduced production and cull value of infected cows and increased replacement costs (Ott et al., 1999). Due to the significant effects on herd productivity, along with the potential public health consequences should MAP be linked to Crohn's disease in humans, voluntary JD control programs have been implemented at the both the national and state levels. Substantial resources have been committed to these control programs, but their success has been difficult to ascertain due to the lack of an efficient monitoring program.

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Recently, the culturing of pooled samples from the herd environment has been investigated as a convenient method for identifying herds infected with MAP. Targeted environmental sampling of manure storage areas and high-traffic cow areas has proven to be a sensitive method (>70%) for identifying herds as infected with MAP (Raizman et al., 2004; Berghaus et al., 2006; Lombard et al., 2006), and has been accepted as an approved method for entry-level testing into the USDA's Voluntary Bovine Johne's Disease Control Program (USDA, 2005a). Environmental sampling has the advantage over other screening methods for dairy herds in that it does not require the handling and testing of individual cattle and is less expensive (Berghaus et al., 2006; Lombard et al., 2006); making it an attractive alternative for monitoring progress of regional or state JD control programs.

In a random survey conducted in 1996, 64% of dairy herds in Michigan were classified as infected with MAP, based on detecting two positive cows on serum ELISA out of a random sample of adult cows older than 2 years of age, proportional to herd size, and designed to detect herds with a minimum 10% within herd JD prevalence. Previous to that study, it was estimated that only 34% of herds were infected with MAP (Johnson-Ifearulundu et al., 1999). The Michigan Voluntary Johne's Disease Control Program (MVJDCP) was implemented in the late 1990s and updated in 2000 (USDA, 2005b). One of the greatest difficulties for the MVJDCP has been determining whether the changes made have been effective in reducing the number of infected herds in the state. The objective of this study was to use targeted environmental sampling of primary manure storage and high-traffic, common cow areas to estimate the prevalence of dairy herds infected with MAP in Michigan. Once determined, periodic statewide monitoring using the same method could be undertaken as a measure of the effectiveness of the MVJDCP.

2. Materials and methods

2.1. Study design

This was a cross-sectional random survey dairy farms in Michigan licensed to sell Grade A milk as defined by the US federal Pasteurized Milk Ordinance (PMO; US Department of Health and Human Services, 2007).

2.2. Sample size determination

It was estimated that 64% of dairy herds in Michigan are infected with MAP (Johnson-Ifearulundu et al., 1999). The sample size was calculated to estimate prevalence of MAP infected dairy herds to within 10% of the actual prevalence with 95% confidence using the following equation (Smith, 1995, pp. 156–157):

$$\text{minimum number of herds to sample} = \frac{P(1-P)Z^2}{d^2}$$

where P is the estimated prevalence of MAP infected herds (0.64), d is the maximum acceptable error between observed and true prevalence (0.1) and Z is the standard normal for 95% confidence (1.96). The calculated minimum number of herds to sample was 86 when adjusted for population size. It was estimated approximately 33% of herd owners might refuse to participate. To account for herds that refused to participate, no longer were in business or could not be contacted, 130 herds were targeted for contact to ensure at least 86 were sampled.

2.3. Herd selection

Because dairy herds vary in size and distribution within the state, a stratified random sampling procedure was used to select a representative sample of herds. The National Agricultural Statistic Service (NASS) has divided Michigan into nine agricultural districts (Fig. 1). Within each district, herds were stratified by size into four categories: 1–99, 100–199, 200–499, and ≥500 cows in accordance with herd size categories established by NASS. The list of licensed Grade A dairy farms was obtained from the Michigan Department of Agriculture; and dairy extension agents and private veterinary practitioners throughout the state were contacted to provide herd size information on as many herds as possible. However, there was still a group of herds for which the size was unknown. Therefore, a fifth stratum for herds of unknown size was added during herd selection. Herds were assigned numbers identifying them by district and stratum (herd size). Using a random number generator for a discrete distribution, a sample proportional to the number of herds in each stratum was selected from each district, with at least one herd in each strata sampled from every district.

Participation in the study was voluntary. A letter describing the project was mailed to each selected herd. A week later an attempt was made to contact each herd by phone. During this phone call it was ascertained whether the owner was willing to participate in the study and, if so, set a date for the herd visit.

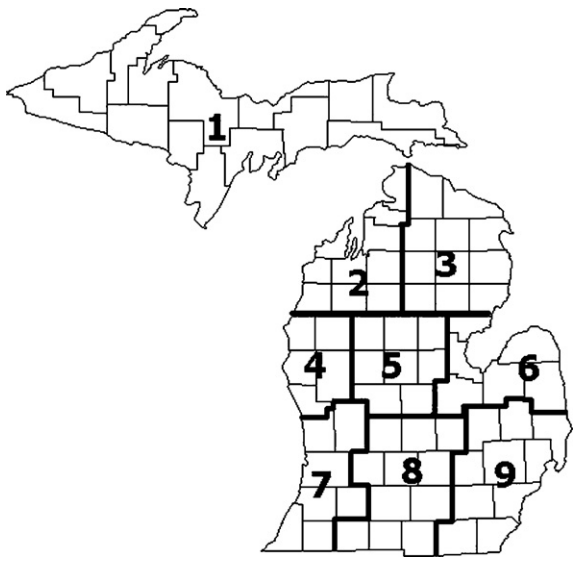

Fig. 1. Michigan agricultural districts.