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

R.B. Pillars [a,b], D.L. Grooms [b,*], J.A. Woltanski [c], E. Blair [b]

[a] Center for Comparative Epidemiology, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA
[b] Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824, USA
[c] USDA, APHIS, Veterinary Services, East Lansing, MI 48823, USA

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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.

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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.

* Corresponding author. Tel.: +1 517 432 1494; fax: +1 517 432 1042.
E-mail addresses: groomsd@cvm.msu.edu, pillarsr@cvm.msu.edu (D.L. Grooms).

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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.

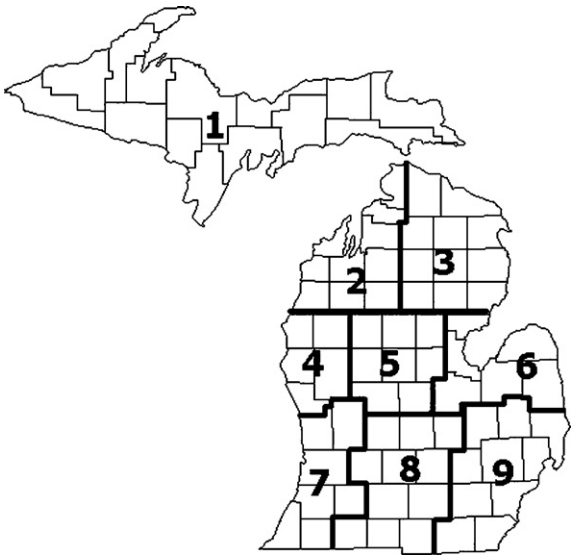

Fig. 1. Michigan agricultural districts.

2.4. Sample collection

One sample each was collected from the primary manure storage/gathering area and a common (high-traffic) cow area from June to August 2006. For liquid or slurry storages, samples were collected 15 cm below the surface from 4 to 6 different locations and pooled to fill a 120 g plastic specimen cup per laboratory sample submission recommendation. For solid manure piles, a core soil sampler was used to collect samples from 10 different locations. The samples were placed in a bucket and mixed thoroughly before filling a 120 g specimen cup. For manure spreaders, a 120 g sample was collected from the beaters (box spreaders) or dispensing area (liquid spreaders). The common cow areas sampled included holding pens, return alleys, free-stall alleys, or gutters depending on the farm. A gloved hand was used to collect 10 random "grab" samples from various locations in the designated area, placed in a bucket and mixed thoroughly before filling a 120 g specimen cup. All samples were shipped on ice to the Diagnostic Center for Population and Animal Health at Michigan State University for MAP culturing.

2.5. Sample culturing

All samples were cultured using the ESP® culture system II (ESP II, Trek Diagnostic Systems, Cleveland, OH). All samples with a positive signal on ESP II prior to 42 days were confirmed as MAP by Kinyoun's acid-fast stain and real-time PCR. All samples with a negative signal on ESP II at 42 days were reported as negative only after testing negative on real-time PCR.

2.6. Questionnaire

A one-page questionnaire was administered to the herd owner or herdsman at the time of sample collection. This questionnaire was used to confirm herd size, obtain previous JD testing history, and information on the purchase of cattle.

2.7. Definition of JD positive herd

A herd was classified as being positive for MAP if at least one of the two environmental samples cultured positive.

2.8. Data analysis

State, agricultural district, and stratum apparent and true prevalence was calculated, as well as the weighted prevalence adjusted for nonresponse. Apparent prevalence was calculated as the number of herds with at least one MAP culture positive sample divided by the total number of herds tested. To account for imperfect test sensitivity, the true prevalence was also calculated using the following equation (Smith, 1995, pp. 81–82): true prevalence = (apparent prevalence + specificity − 100%)/(sensitivity + specificity − 100%). The sensitivity and specificity used for the proposed sampling protocol at the herd level was 81% and 100% respectively based on previous research by the authors, using the same sampling procedure (Pillars et al., 2009). The sensitivity of targeted environmental sampling for MAP on dairy farms reported by other studies ranges from 74% (Berghaus et al., 2006) to 90% (Raizman et al., 2004), despite slightly different sampling protocols being used in each study. Furthermore, the estimated 81% sensitivity used in this study was in close agreement with the 2002 NAHMS Johne's disease on dairy operations survey, where the sensitivity of environmental sampling for identifying MAP infected herds in the Midwest was 83.3% (Lombard et al., 2006). To remove bias caused by nonresponse, the weighted prevalence was also calculated. The weighting factor within each stratum was adjusted for herds that were selected to be sampled, but could not be contacted or refused to participate (nonresponders). The weighted prevalence was then calculated as the product of the number of herds with a positive culture multiplied by the adjusted weighting factor divided by the total number of herds tested in each respective stratum (Lohr, 1999, pp. 265–268).

Logistic regression was used to test the statistically significant differences between herds that agreed to participate in the study and those that refused to participate in terms of agricultural district and stratum. Univariable logistic regression was used to compare the following parameters between herds classified as positive for MAP to those classified as negative: agricultural district, actual herd size, history of testing for JD in past 5 years, history of purchasing cows in past 5 years, type of manure storage, and type of common cow area sampled. A multivariable model was then built using purposeful selection. Variables found statistically significant on univariable analysis, and their interactions, were added to the model one by one keeping only those with statistically significant p-values. For all analyses, a p-value of <0.05 was considered statistically significant. All logistic analysis was performed using PROC LOGISTIC (SAS version 9.1, SAS institute, Inc., Cary, NC, USA).

3. Results

3.1. Herd participation

A total of 127 herds were contacted to participate in the study. Thirty-three herds (26%) refused to participate and 94 herds were tested. Reasons for not participating included: not being interested, no longer in business, and herds ($n = 3$) we were unable to contact by phone despite several attempts. There was no statistical difference between herds that participated in the study and those that did not in terms of distribution across agricultural districts ($p = 0.49$) or stratum ($p = 0.84$); although it should be noted that all herds contacted with >500 cows agreed to participate.

3.2. Prevalence

Of the 94 herds that were surveyed, 38 (40.4%) had at least one MAP positive environmental culture. The apparent, true, and weighted prevalence by agricultural district and stratum (herd size) are shown in Tables 1 and 2 respectively. All herds tested with >200 cows were classified as positive for MAP.

Table 1
Herd level apparent, true, and weighted prevalence of *Mycobacterium avium paratuberculosis* (MAP) by agricultural district.

District	No. herds tested	No. herds positive	Apparent prevalence (%)	True prevalence (%)	Weighted prevalence (%)
1	5	3	60.0	73.2	110.4
2	3	2	66.7	81.3	164.7
3	8	2	25.0	30.5	33.5
4	9	5	55.6	67.8	61.1
5	13	4	30.8	37.5	42.8
6	15	5	33.3	40.7	43.0
7	12	5	41.7	50.8	45.0
8	19	7	36.8	44.9	50.8
9	10	5	50.0	61.0	54.5
Statewide (95% CI)	94	38	40.4 (35.4–45.5)	49.9 (44.8–55.1)	54.6 (49.4–59.7)

Table 2
Herd level apparent, true, and weighted prevalence of *Mycobacterium avium paratuberculosis* (MAP) by stratum (herd size).

Stratum (herd size)	No. herds tested	No. herds positive	Apparent prevalence (%)	True prevalence (%)	Weighted prevalence (%)
1–99 cows	65	19	29.2	35.6	37.7
100–199 cows	14	4	28.6	34.8	38.9
200–499 cows	6	6	100.0	100.0	165.0
>500 cows	9	9	100.0	100.0	100.0
Statewide (95% CI)	94	38	40.4 (35.4–45.5)	49.9 (44.8–55.1)	54.6 (49.4–59.7)

Table 3
Univariable analysis of parameters for *Mycobacterium avium paratuberculosis* (MAP) positive herds.

Parameter	*p*-Value (Wald Chi-Square)	OR	95% CI	
			Lower	Upper
Herd size (per 100 cows)	0.0022	2.80	1.45	5.42
Tested for MAP in past 5 years (yes)	<0.0001	6.36	2.55	15.85
Purchased cows in past 5 years (yes)	0.0017	4.76	1.80	12.59
Type of manure storage (slurry)	0.0583	2.66	0.97	7.32

Table 4
Final multivariable model for *Mycobacterium avium paratuberculosis* (MAP) positive herds.

Parameter	*p*-Value (Wald Chi-Square)	OR	95% CI	
			Lower	Upper
Herd size (per 100 cows)	0.0281	2.24	1.09	4.60
Tested for MAP in past 5 years (yes)	0.0050	4.62	1.59	13.47
Purchased cows in past 5 years (yes)	0.0480	3.09	1.01	9.47

AIC = 92.446, -2 Log L = 84.446.

3.3. Logistic regression

Using MAP positive herds as the referent group, univariable analysis revealed no statistically significant difference between MAP positive and negative herds in terms of agricultural district ($p = 0.82$) or type of common cow area sampled ($p = 0.57$). Type of manure storage area sampled approached significance ($p = 0.06$) with herds with lagoons, or similar types of manure slurry holding areas, being more likely to be culture positive for MAP (OR = 2.66; 95% CI: 0.966, 7.321). Univariable analysis was not possible on stratum because, as demonstrated in Table 2, there were no test negative herds in the two strata with >200 cows, resulting in questionable model validity. In its place, the actual herd size (a continuous variable) was used. Herd size, along with testing for MAP in the past 5 years, and purchasing cows in the past 5 years were all statistically significant on univariable analysis (Table 3). Despite investigating all possible interactions, the final multivariable model included only the three main effect variables (Table 4).

4. Discussion

Based on this study, the apparent prevalence of dairy herds infected with MAP in Michigan is 40.4% with a calculated true prevalence of 49.9%, and weighted prevalence of 54.6%. This is lower than estimated in a previous Michigan study, 64%, based on the random testing of adult cattle using serum ELISA and ≥2 cows testing positive (Johnson-Ifearulundu et al., 1999). However, given the 95% confidence intervals calculated in this study and a different testing protocol, it cannot be definitively stated that there are fewer dairy herds infected with MAP in

Michigan now as compared to 10 years ago when that study was performed. Infected herds were found in all agricultural districts throughout the state, with no significant differences between districts, suggesting that JD is equally distributed across the state. Based on herd size, the distribution of the herds selected for this study was proportional to, and reflected the distribution of dairy herds in the Michigan. However, due to refusal to participate (all 33 herds that refused to participate fell in the smaller three strata), the distribution of herds actually tested was biased towards larger herds, which appear to be at greater risk of being infected with JD. To correct for this bias, the weighted prevalence was calculated.

It is certainly possible that some infected herds were misclassified as "negative" using the sampling protocol described, but misclassification would only occur in herds with extremely low prevalence (<7%) of fecal shedding (Pillars et al., 2009). It was assumed specificity was 100%, as it would be unlikely a herd with no MAP infected cattle would have a positive environmental sample.

The protocol used in this study provided the best sensitivity (81%) for the least cost ($30/culture or $60/herd). Environmental sampling protocols reported in other studies involved culturing 3–5 samples with sensitivities ranging from 70.4% to 90% (Raizman et al., 2004; Berghaus et al., 2006; Lombard et al., 2006) with equivalent costs of $90–150. Using the protocol with 90% sensitivity (Raizman et al., 2004), an improvement in sensitivity of only 8%, would have doubled the cost ($120/herd) of this study. We wanted an efficient and economical protocol that could be used for routine surveillance of herd MAP prevalence at the state level. In other words, the purpose of our testing was to identify MAP infected herds. While we hoped to have as little herd misclassification as possible in this study, the penalty for misclassifying a herd as negative was not considered worth the extra cost of more extensive sampling. In contrast, the environmental sampling protocol outlined in the USDA's Johne's Program Standards involves collecting a total of six samples and would currently cost $180/herd in Michigan. The purpose of the USDA's protocol (assuming all samples are negative) is to identify uninfected herds with 85% confidence for entry into a JD certification program (USDA, 2005a). The penalty for misclassifying a MAP infected herd as uninfected is much higher in that instance, and therefore the additional testing and cost is justified.

In-depth analysis of risk factors associated with a herd being positive for MAP was beyond the scope of this study. It was assumed if prior testing for MAP had occurred, there was a higher probability that cattle on the farm were infected. Thirty-eight (40%) of the 94 herds sampled had tested for MAP during the past 5 years, with positive herds being 4.6 times more likely to have tested for MAP than negative herds. This was similar to findings of a previous study (Obasanjo et al., 1997).

In this study, herds that had purchased cattle within the previous 5 years were approximately three times more likely to be MAP positive. The purchase of infected cattle is considered the primary mode of JD transmission between herds (Sweeney, 1996). Analysis of the NAHMS Dairy' 96 data found that herds diagnosed with JD were more likely to buy cattle (Wells and Wagner, 2000). When herds expand, they tend to do so through the purchase of cattle; and unfortunately, rarely is any consideration given to the MAP status of the herd of origin. Furthermore, larger herds were more likely to purchase cattle than smaller herds (USDA, 2002). Thus, it follows that larger herds, as compared to smaller herds, are more likely to be infected; and infected herds are more likely to have purchased cattle in the past 5 years.

Finally, there was a tendency towards significance for MAP positive herds to have a lagoon or similar type of manure slurry holding system. These types of manure storage areas are common on large farms, which are more likely to be infected. It is also possible that the liquid to semi-liquid nature of the manure in lagoons results in more thorough mixing and even dispersal of MAP than occurs in manure piles, thereby increasing the likelihood of collecting a "positive" sample. Conversely, smaller farms that use solid manure storage systems might have been misclassified as uninfected with MAP due to a lack of fecal mixing, resulting in a decreased likelihood of collecting a contaminated sample.

5. Conclusion

The apparent prevalence of Michigan dairy herds infected with MAP was 40.4%, with a true prevalence of 49.9%, and weighted prevalence of 54.6% based on environmental sampling of the primary manure storage area and a high-traffic, common cow area. This is likely a conservative estimate, as the sampling protocol used in this study may have misclassified herds with a within herd infection rate of <7% as negative.

The environmental sampling protocol used in this study is an economically attractive alternative for monitoring herd level prevalence and the progress of JD control programs at the state or national level. Implementation of such a program would aid states in monitoring JD control program progress and guide changes over time.

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