Prevalence of Dairy Herds Infected with Johne's Disease in Michigan as determined by Environmental Sampling

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Abstract

Objective—Estimate the prevalence of dairy herds infected with Johne's disease (JD) in Michigan.

Design—Cross-sectional, stratified random survey.

Sampled population—94 randomly selected dairy herds.

Procedure—One sample each from the primary manure storage area and a common cow area from each herd was cultured for *Mycobacterium avium paratuberculosis* (MAP) using the Trek ESP culture system. A herd was classified as infected with Johne's disease if at least one sample cultured was positive for MAP. State, agricultural district, and stratum apparent and true prevalence was calculated. Summary information on past JD testing and cattle purchase history was also collected from each farm. Logistic regression was performed to determine their importance on the JD status of the herd.

Results—38 (40.4%) of 94 herds were positive for JD. Herds that had tested for JD or purchased cattle in the previous five years were 6.2 and 4.6 times, respectively, more likely to be infected that herds that had not.

Conclusions and Clinical Relevance—The environmental sampling protocol used in this study was an effective method for estimating the prevalence of MAP infected dairy herds in Michigan at 1/3 the cost of the environmental sampling protocol approved by the USDA for entry-level screening of dairy herds into the Voluntary Bovine Johne's Disease Control Program. The protocol used in this study is an economically attractive

alternative for monitoring the progress of JD control programs at the state, or regional, as well as individual herd levels.

Introduction

Johne's Disease (JD) is a 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 JD in the US to be 21.6% [1], but other estimates range from 21-93% depending on region and testing method used to classify infected herds[2-7]. Johne's disease costs the US dairy industry an estimated $200-250 million annually due primarily to reduced production and cull value of infected cows and increased replacement costs[8]. Due to the significant effects on herd productivity along with the potential public health consequences should MAP be linked to Crohn's Disease in people, 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 accurate monitoring program.

Recently "targeted" environmental sampling of manure storage areas and high traffic cow areas has proven to be greater than 70% effective in identifying herds as infected with MAP[9-12] and has been accepted as an approved method for entry-level testing into the USDA's Voluntary Bovine Johne's Disease Control Program[13]. 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 [9, 10]. It also allows estimation of the within-herd fecal prevalence[9, 12, 14], making it an attractive alternative for monitoring progress of regional or state JD control programs, as well as individual herd JD control programs.

In a random survey conducted in 1996, 64% of the dairy herds in Michigan were classified infected with JD based on testing 30 randomly selected cows. Previous to that study, it was estimated that only 34% of herds were infected with MAP[15]. The Michigan Voluntary Johne's Disease Control Program (MVJDCP) was implemented in the late 1990's and was updated in 2000[16]. One of the biggest 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 JD in Michigan.**

Materials and Methods

Study design. Cross-sectional random survey of licensed Grade A dairy farms in Michigan.

Sample size determination. It was estimated that 64% of dairy herds in Michigan are infected with MAP[15]. 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[17]:

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

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. It was assumed that up to 25% of herd owners would refuse to participate. Therefore, a target of 120 herds was selected to be contacted to ensure that at least 86 are sampled.

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. Within each district, herds were stratified by size into four categories: 1-99, 100-199, 200-499, and >500. The list of licensed Grade A dairy farms was obtained from the Michigan Department of Agriculture. Herd size was not available on this list, so dairy extension agents and private practitioners throughout the state were contacted to provide herd size information on as many herds as possible. However, there was still a substantial 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. 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 or not the owner was willing to participate in the study and, if so, set a date for the herd visit.

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 6 inches below the surface from 4-6 different locations and pooled to fill an 8 oz specimen cup. For solid manure piles, a core sampler was used to collect samples from 10 different locations. The samples were placed in a bucket and mixed thoroughly before filling an 8 oz specimen cup. For manure spreaders, an 8 oz 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 an 8 oz specimen cup. All samples were shipped on ice to the Diagnostic Center for Population and Animal Health at Michigan State University, East Lansing, MI for MAP culturing.

Sample culturing. All samples were cultured using the Trek ESP culture system II[a]. All samples testing positive on Trek prior to 42 days were confirmed as MAP by acid-fast staining and PCR. All samples negative on Trek at 42 days were reported as negative only after testing negative on PCR.

[a] Trek Diagnostic Systems, Cleveland, OH, USA

Questionnaire. A one-page questionnaire was administered to the herd owner or herdsman at the time of sample collection. This survey was used to confirm herd size as well as obtain previous JD testing history on the farm and information on if or when cattle had been purchased in the past.

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

Data analysis. State, agricultural district, and stratum apparent and true prevalence was calculated. 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[17]:

$$True\ prevalence = \frac{Apparent\ prevalence + Specificity - 100\%}{Sensitivity + Specificity - 100\%}$$

The sensitivity and specificity used for the proposed sampling protocol at the herd level was 84% and 100% respectively based on preliminary research by Michigan State University[11].

Logistic regression was used to test for statistically significant differences between herds that agreed to participate and those that did not in terms of agricultural district and stratum. Univariate logistic regression was used compare the following parameters between herds classified as positive for JD 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 multivariate model was then built including only those variables found statistically significant on univariate analysis. Purposeful selection was used based on plausible associative pathways, with variables (and their interactions) eliminated one by one until only those with statistically significant p-values remained. For all analyses, a p-value of <0.05 was considered significant. All logistic analysis was performed using commercially available software.[b]

Results

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 two herds 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 no herds with >500 cows refused to participate.

Prevalence. Of the 94 herds that were surveyed, 38 (40.4%) were infected with JD. The apparent and true prevalence by agricultural district and stratum are shown in Tables 1 and 2 respectively. All herds tested with >200 cows were classified as positive for JD.

[b] PROC LOGISTIC, SAS version 9.1, SAS Institute, Inc., Cary, NC, USA

Table 1: Herd level apparent and true Johne's Disease prevalence by agricultural district				
District	No. Herds Tested	No. of herds Positive	Apparent Prevalence (%)	True Prevalence (%)
1	5	3	60.0	71.4
2	3	2	66.7	79.4
3	8	2	25.0	29.8
4	9	5	55.6	66.1
5	13	4	30.8	36.6
6	15	5	33.3	39.7
7	12	5	41.7	49.6
8	19	7	36.8	43.9
9	10	5	50.0	59.5
State	94	38	40.4	48.1

Table 2: Herd level apparent and true Johne's Disease prevalence by Stratum				
Stratum (herd size)	No. Herds Tested	No. of herds Positive	Apparent Prevalence (%)	True Prevalence (%)
1-99 cows	65	19	29.2	34.8
100-199 cows	14	4	28.6	34.0
200-499 cows	6	6	100.0	100.0
>500 cows	9	9	100.0	100.0
State	94	38	40.4	48.1

Logistic regression. Using JD positive herds as the referent group, univariate analysis revealed no statistical difference between JD 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). Univariate 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 JD in the past 5 years, and purchasing cows in the past 5 years were all significant on univariate analysis. The p-values, OR and 95% CI for the OR are shown in Table 3.

Table 3: Univariate analysis of significant parameters. Referent group is JD positive herds.

Parameter	p-value (Wald Chi-Square)	OR	95% CI (OR)	
Herd size (per 100 cows)	0.0022	2.26	1.17	4.37
Tested for JD 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

In analyzing plausible pathways by which the three remaining variables could be associated with the JD status of a herd, it was determined that herd size acted as an intervening variable for purchased cows in the past 5 years. As such it was dropped from the analysis. There was no significant interaction between the two remaining variables. The final multivariate model consisted of tested for JD in past 5 years (OR=6.18, 95% CI: 2.35, 16.26) and purchased cows in past 5 years (OR=4.58, 95% CI: 1.59, 13.20).

Discussion

Based on this random study, the apparent prevalence of dairy herds infected with JD in Michigan is 40.4% with a calculated true prevalence of 48.1%, and a margin of error ± 10%. 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.

The distribution of herd size of the herds selected for this study was proportional 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. Therefore the prevalence estimated in this study may be higher than the real prevalence of JD infected herds.

Because the true prevalence of our study was only 48% compared to the estimated prevalence of 64% used to calculate our sample size, the question arose as to whether the 94 herds tested provided enough power to keep us within our 10% margin of error. To see what our margin of error actually was, the sample size equation was solved for d, using a sample size of 94 and P=0.48. The calculated value of d was 0.102, suggesting that we did remain within our desired margin of error.

The environmental sampling protocol used in this study was different from that approved by the USDA as an entry-level screening test. The USDA's Johne's Program Standards states that "two environmental samples from each of the following locations on the farm: manure concentration areas (cow housing alleyways or gutters), manure storage areas (lagoons, piles, pits, or manure spreader), and another manure concentration area (sick cow pens or other cow alleyways and travel-ways)", for a total of six samples, should be submitted for culture. The other approved method for screening dairy herds is performing an ELISA test on 30 randomly selected cows 36 months of age or older[13]. In Michigan the cost for Johne's culture is $30/sample and that for ELISA is $6/sample. Thus, using the USDA's approved environmental sampling protocol, there is no economic advantage over ELISA testing. Furthermore, from a monitoring perspective at the state level, $180/herd is likely to be cost prohibitive for routine use.

In Michigan there is an ongoing longitudinal study following the progress of JD control programs on infected dairy farms. Part of that program involves culturing several different areas on the farms to monitor the distribution of MAP in the environment and track how it changes as within herd prevalence changes. Based on preliminary results of that project, and using the environmental sampling protocol used in this study, MAP was isolated from the manure storage area and/or common cow area 84% of the time when within herd prevalence (based on whole herd fecal culture) was >2%. Specificity of this protocol was 100% [11]. With regards to this study, it would be unlikely to culture MAP from an uninfected herd. Thus, screening dairy herds with one sample each from the primary manure storage area and a high-traffic, common cow area is comparable to the protocol approved by the USDA for identifying JD infected herds and costs one third as much.

In-depth analysis of risk factors associated with a herd being positive for JD was beyond the scope of this study. Through the study questionnaire, summary information on recent herd additions and previous JD testing history was obtained. Previous JD testing was an attempt to objectively ascertain the likelihood that a herd was infected with JD. It was assumed that if prior JD testing had occurred, there was a high probability that cattle on the farm were infected. Thirty-eight (40%) of the 94 herds sampled had tested for JD during the past five years, with positive herds being over six times more likely to have tested for JD than negative herds. This was similar to findings of a previous study[6].

In this study, herds that had purchased cattle within the previous five years were over 4.5 times more likely to be JD positive. The purchase of infected cattle is considered the primary mode of JD transmission between herds[18]. Analysis of the NAHMS Dairy ’96 study found that herds diagnosed with JD were more likely to buy cattle[19]. When herds expand, they tend to do so through the purchase of cattle, and unfortunately rarely is any consideration given to the JD status of the herd of origin. Furthermore, larger herds were more likely to purchase cattle than smaller herds[20]. Thus, if 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 five years.

Finally, there was a tendency towards significance for JD positive herds to have a lagoon or similar type of manure slurry holding system. Of course these types of manure storage areas are common on large farms which are more likely to be infected. It is also possible 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.

Conclusion

The apparent prevalence of Michigan dairy herds infected with JD was 40.4%, with a true prevalence of 48.1% based on environmental sampling of the primary manure storage area and a high-traffic, common cow area. All herds tested with >200 cows were positive for JD. Positive herds were also more likely to have tested for JD and purchased cattle in the past five years. While the environmental sampling protocol used in this study did not adhere to that approved by the USDA for the Voluntary Bovine Johne's Control Program, its performance for screening dairy herds for JD is comparable and it costs 66% less. This makes it a more attractive alternative for monitoring the progress of JD control programs at the state, or regional, level as well as the individual herd level.

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