Sensitivity of test strategies used in the Voluntary Johne’s Disease Herd Status Program for detection of *Mycobacterium paratuberculosis* infection in dairy cattle herds

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**Objective**—To evaluate sensitivities at the herd level of test strategies used in the Voluntary Johne’s Disease Herd Status Program (VJDHSP) and alternative test strategies for detecting dairy cattle herds infected with *Mycobacterium paratuberculosis*.

**Design**—Nonrandom cross-sectional study.

**Sample Population**—64 dairy herds from Pennsylvania, Minnesota, Colorado, Ohio, and Wisconsin. Fifty-six herds had at least 1 cow shedding *M. paratuberculosis* in feces; the other 8 herds were free from paratuberculosis.

**Procedure**—For all adult cows in each herd, serum samples were tested for antibodies to *M. paratuberculosis* with an ELISA, and fecal samples were submitted for bacterial culture for *M. paratuberculosis*. Sensitivities at the herd level (probability of detecting infected herd) of various testing strategies were then evaluated.

**Results**—Sensitivity at the herd level of the testing strategy used in level 1 of the VJDHSP (use of the ELISA to test samples from 30 cows followed by confirmatory bacterial culture of feces from cows with positive ELISA result) ranged from 33 to 84% for infected herds, depending on percentage of cows in the herd with positive bacterial culture results. If follow-up bacterial culture was not used to confirm positive ELISA results, sensitivity ranged from 70 to 93%, but probability of identifying uninfected herds as infected was 89%.

**Conclusions and Clinical Relevance**—Results suggest that the testing strategy used in the VJDHSP will fail to identify as infected most dairy herds with a low prevalence of paratuberculosis. A higher percentage of infected herds was detected if follow-up bacterial culture was not used, but this test strategy was associated with a high probability of misclassifying uninfected herds. (J Am Vet Med Assoc 2002;220:1053–1057)

Paratuberculosis (Johne’s disease) is a chronic disease of cattle and other ruminants worldwide. Infection of young calves with the causative organism, *Mycobacterium paratuberculosis (M. avium subsp paratuberculosis)*, results in a slow progression of the disease, with clinical signs most frequently seen in cows between 3 and 6 years old. Cows in advanced stages of the disease develop diarrhea, lose weight, and have a substantial decrease in milk production. There is no known effective approved treatment, and affected cows are typically sold for slaughter. Transmission is primarily through the fecal-oral route and via colostrum, milk, the placenta, and environmental contamination.

On dairy cattle operations, economic losses attributed to paratuberculosis include premature culling, reduced milk production, and reduced body weight of cows that are slaughtered. The National Animal Health Monitoring System has estimated that the annual cost of paratuberculosis in infected US dairy operations is approximately $100/cow, with annual costs > $200/cow in herds with a high prevalence of infection. It has also estimated that at least 22% of dairy herds in the United States have cattle infected with *M. paratuberculosis*. The continuing expansion of dairy herds and greater movement of dairy cattle across the United States has facilitated the introduction of the causative organism to previously uninfected herds. In addition, concern has arisen that *M. paratuberculosis* may be 1 of the causes of Crohn’s disease in humans.

In response to these animal health issues and potential public health concerns, a Voluntary Johne’s Disease Herd Status Program (VJDHSP) for identifying herds free from paratuberculosis has been developed. Under this program, a subset of the adult cattle in a herd is tested with an ELISA to detect antibodies to *M. paratuberculosis*. At the first level of the program, a random sample of 30 cows in their second or greater lactation is tested, and advancement to higher levels of the program requires annual testing first with the ELISA and later by means of bacterial culture of individual fecal samples from a much larger sample of cows in the herd or from all cows in the herd for herds with...
≤ 600 cows in their second or greater lactation. The ELISA is used in the early stages of the program because of its relatively low cost and quick turnaround time, even though test sensitivity in subclinically infected cows is reportedly low (15 to 75%) and test specificity is < 100%. The program accounts for the imperfect specificity of the ELISA during any herd test by allowing confirmation of positive ELISA results through bacterial culture of fecal samples, although this strategy reduces herd sensitivity. Current methods for bacterial culture of fecal samples from individual cows are associated with higher test sensitivity, particularly in subclinically infected cattle, and 100% test specificity, but these benefits come at a higher cost, and results may not be available for up to 16 weeks. Thus, although it is far from ideal, the VJDHSP is considered the best compromise between scientific validity and cost-benefit ratio, using tests currently available.

The VJDHSP has been endorsed by the United States Animal Health Association as a uniform model for state programs, and several states have implemented the VJDHSP. However, the herd sensitivity of this program has not been validated with data from cattle herds. In addition, a few states have implemented a modified program that does not include use of bacterial culture to confirm positive ELISA results. The purposes of the study reported here, therefore, were to evaluate herd sensitivity of test strategies used in the VJDHSP, to compare sensitivities with sensitivities of alternative methods for detecting dairy cattle herds infected with M paratuberculosis, and to evaluate factors associated with herd sensitivity, using data from dairy cattle herds.

**Materials and Methods**

Sixty-four dairy herds (29 in Pennsylvania, 22 in Minnesota, 7 in Ohio, 4 in Colorado, and 2 in Wisconsin) were used in the study. The 29 herds in Pennsylvania had been tested for paratuberculosis at the University of Pennsylvania Johne’s Laboratory by means of bacterial culture of fecal samples from all cows that had calved at least once. Fecal samples for bacterial culture were initially collected between 1987 and 1999 because of owner concerns about the possibility of paratuberculosis in the herd. Nine of these herds were selected for inclusion in the present study because prevalence of paratuberculosis, determined on the basis of results of bacterial culture of fecal samples, had previously been found to be ≥ 10%, and 10 were selected because prevalence had previously been found to be < 10%. For the remaining 10 herds, this was the first time fecal samples had been collected for bacterial culture. This group of 10 herds was selected for the study because of our concern that herd sensitivity could be lower in herds tested previously if cows with positive test results were culled by herd managers as part of a herd control program. For all 29 Pennsylvania herds, results of bacterial culture of fecal samples from the entire adult herd were positive for at least 1 cow. Sera had been collected from all cows in these herds at the time fecal samples had been collected and stored at −20 C. For the present study, sera were tested for antibodies to M paratuberculosis at the University of Pennsylvania Johne’s Laboratory during March and April 2000 (except for 1 herd for which sera were tested during July 1999), using a commercially available ELISA.

For the 35 other herds included in the study, bacterial culture of fecal samples and serologic testing were performed as part of other research efforts at veterinary diagnostic laboratories in each state. Fecal samples were collected from all adult cows in all herds, with the exception of 1 herd for which fecal samples were collected from 498 of 1,800 cows. For 27 of these herds, results of bacterial culture of fecal samples were positive for at least 1 cow; samples had been collected from these herds because of owner concern about clinical paratuberculosis among cows in the herd. For the remaining 8 herds, results of bacterial culture were negative for all cows; samples had been collected from these herds to verify that they were free from paratuberculosis, and clinical paratuberculosis had not been diagnosed before or after collection of samples.

The diagnostic laboratories involved in testing samples from these herds all used similar methods for bacterial culture of fecal samples for M paratuberculosis, including use of Herrold egg yolk medium, although specific culture methods differed slightly among laboratories. All laboratories used the same commercially available ELISA for serologic testing.

**Data analysis**—Herd-level parameters evaluated in the present study included sample size (30, 40, or 50 samples/herd or all adult cows in the herd), whether this was the first time testing had been performed, the percentage of positive bacterial culture results (≤ 4.9%, 5 to 9.9%, or ≥ 10%), the percentage of positive ELISA results (≤ 4.9%, 5 to 9.9%, or ≥ 10%), and herd size (≤ 49 cows, 50 to 99 cows, 100 to 199 cows, or ≥ 200 cows). Although all cows in the study herds were tested (except for 1 large herd), we estimated the probability of detecting paratuberculosis in the herd if only a finite number of samples were collected (30, 40, or 50 samples/herd), using a hypergeometric distribution function to account for limited sample size. Calculations were performed with commercially available software. Herds were considered to have paratuberculosis if results of bacterial culture of fecal samples were positive for at least 1 cow. Herd sensitivity was defined as the probability (multiplied by 100 to create a percentage) that at least 1 cow in a herd with paratuberculosis would have positive results for both the ELISA and bacterial culture. This corresponded to the method used in the VJDHSP to identify paratuberculosis in herds (ie, use of the ELISA to test a sample of adult cows in the herd followed by bacterial culture of fecal samples from any cows with positive ELISA results). Herd sensitivity was calculated as 1 minus the probability (multiplied by 100) that no cow with positive ELISA and bacterial culture results would be detected. To evaluate the sensitivity of using the ELISA alone, without the use of bacterial culture of fecal samples for follow-up confirmatory testing of positive results, herd sensitivity was defined as the probability (multiplied by 100) that at least 1 cow in a herd with paratuberculosis would have positive ELISA results without regard to results of bacterial culture, and analyses were repeated.

For each sample size (30, 40, or 50 samples/herd or all adult animals in the herd), herd sensitivity was compared among herds grouped on the basis of percentage of positive bacterial culture results, percentage of positive ELISA results, and herd size, using generalized linear model procedures. Scheffe tests were used for multiple pairwise comparisons. Herd sensitivity was compared among sample sizes with a mixed model procedure, with the estimated probability of herd detection as the outcome variable and herd as a random effect variable to account for clustering of data by herd. Descriptive and inferential statistics were calculated with commercially available software.

**Results**

The 29 dairy herds in Pennsylvania consisted of 25 and 197 adult cows. All 29 had a history of paratuberculosis in the herd, and results of bacterial
culture of fecal samples were positive for *M. paratuberculosis* for at least 1 cow from each herd. Herd sensitivity for detecting paratuberculosis (ie, probability that at least 1 cow in a herd with paratuberculosis would have positive results for both the ELISA and bacterial culture) for the 10 herds for which fecal samples had been collected for the first time (mean ± SE probability of detection, 50 ± 17%) was not higher than herd sensitivity for the 9 herds previously found to have a ≥10% prevalence of paratuberculosis (100 ± 0%) or herd sensitivity for the 10 herds previously found to have a <10% prevalence of paratuberculosis (80 ± 13%). For 4 of the 10 herds from which fecal samples had been collected for the first time, bacterial culture results were positive for ≤4.9% of the adult cows in the herd. For 1 herd, bacterial culture results were positive for between 5 and 9% of the adult cows in the herd, and for 5 herds, bacterial culture results were positive for >10% of the adult cows in the herd. The 35 dairy herds in the study from states other than Pennsylvania consisted of between 31 and 498 adult cows. For 27 of these herds, results of bacterial culture of fecal samples were positive for at least 1 cow from each herd. Herd sensitivity for these 27 herds was similar to sensitivities for the 29 Pennsylvania herds; therefore, results from all herds were combined for all subsequent analyses. The 56 herds with paratuberculosis consisted of between 25 and 498 adult cows (mean, 116 cows). The predominant breed was Holstein. Information on the age of the cows at the time samples were collected was not available for most herds. Overall, 6,504 cows were tested from the 56 herds with at least 1 cow with positive bacterial culture results, using both the ELISA and bacterial culture of a fecal sample. Of these, 582 (9%) had positive bacterial culture results, and 5,922 had negative bacterial culture results. Of the cows with positive bacterial culture results, 210 (36%) had positive ELISA results. In herds with negative bacterial culture results, 518 cows were tested, and 63 (12%) cows had positive ELISA results. For herds with the lowest percentage of cows with positive bacterial culture results (0.1 to 4.9%), collecting samples from 30 cows/herd resulted in a herd sensitivity of 33% (ie, probability [multiplied by 100] that at least 1 cow in a herd with paratuberculosis would have positive results for both the ELISA and bacterial culture; Table 1). For herds with higher proportions of cows with positive bacterial culture results, collecting samples from 30 cows/herd resulted in higher herd sensitivities, and herd sensitivity was significantly lower for herds in which ≤4.9% of cows had positive bacterial culture results than for herds in which ≥5% of cows had positive bacterial culture results. However, when all adult cows were tested, herd sensitivity did not vary significantly with percentage of cows in the herd with positive bacterial culture results. Similarly, herd sensitivity was significantly lower for herds in which ≤4.9% of cows had positive ELISA results than for herds in which ≥5% of cows had positive ELISA results when samples were collected from 30, 40, or 50 cows/herd (Table 1). However, when all adult cows were tested, herd sensitivity did not vary significantly with percentage of cows in the herd with positive ELISA results. Also, herd sensitivity did not vary significantly with herd size, regardless of whether samples were collected from 30, 40, or 50 cows/herd or all adult cows in the herd. Herd sensitivity for all herds overall was lower when samples were collected from 30 cows/herd (61%) than when samples were collected from 40 cows/herd (68%), 50 cows/herd (72%), or all cows in the herd (86%; Table 1). Subjectively, the effect of sample size on herd sensitivity appeared to be greater for herds with a low prevalence of paratuberculosis (≤4.9% of cows with positive bacterial culture results) than for herds with higher prevalences of paratuberculosis. A herd sensitivity of 86% when collecting samples from all adult cows in the herd means that this method of testing failed to detect 14% of infected herds. When herd sensitivity was defined as the probabil-

| Table 1—Herd sensitivity of using a commercial ELISA followed by confirmatory bacterial culture of fecal samples from cows with positive ELISA results for detection of paratuberculosis in dairy cattle herds |
|---|---|---|---|---|
| Variable | No. of herds | 30 | 40 | 50 |
| All herds | 56 | 61% (5%) | 68% (5%) | 72% (5%) | 86% (5%) |
| Percentage of cows with positive bacterial culture results | 0.1 to 4.9 | 22 | 40% (8%) | 45% (9%) | 73% (10%) |
| | 5.0 to 9.9 | 11 | 68% (8%) | 84% (9%) | 91% (9%) |
| | ≥ 10 | 23 | 91% (4%) | 93% (4%) | 96% (4%) |
| Modal R | 0.43 | 0.39 | 0.34 | 0.09 |
| Percentage of cows with positive ELISA results | 0.1 to 4.9 | 18 | 36% (8%) | 44% (10%) | 72% (11%) |
| | 5.0 to 9.9 | 18 | 79% (7%) | 83% (7%) | 89% (8%) |
| | ≥ 10 | 20 | 80% (7%) | 84% (7%) | 95% (5%) |
| Modal R | 0.22 | 0.20 | 0.18 | 0.08 |
| Herd size (No. of adult cows) | 25 to 49 | 20 | 68% (9%) | 74% (10%) | 75% (10%) |
| | 50 to 99 | 17 | 68% (9%) | 75% (9%) | 82% (10%) |
| | ≥ 100 | 19 | 54% (7%) | 62% (7%) | 67% (8%) |

Data are given as probability multiplied by 100 (SE) of identifying at least 1 cow with positive ELISA and bacterial culture results.

"For each sample size group, values for individual categories were significantly (P < 0.05) different if they had different superscript letters."
Ruminants

about the disease. Herds without paratuberculosis were

(positive bacterial culture results had a history of clini-

culture of fecal samples for

to identify herd status and the

domly selected, and all herds with at least 1 cow with posi-

table culture results had a history of clinical paratuberculosis. Infected dairy herds were selected

on the basis of this known herd infection status and the

fecting competency in test performance.

Herds included in the present study were not ran-

nor with paratuberculosis with currently available tests. This evalua-

tests did not always agree for individual
ty (multiplied by 100) that at least 1 cow in a herd

r group with at least 1 cow with positive ELISA results, for detection of paratuberculosis in

tes (8/121, 1/65, 14/56, 5/98, 2/40, 2/32, 14/48, and 17/58 cows), even though results of

infection status. Accurate identification of herd infec-

s to having paratuberculosis) ranged from 89 to

 lâus, and geographic locations and were typical of

tates was compiled. All laboratories used the same

els were calculated for specific paratuberculosis prevalences and herd sizes rather than as a single value for all herds. Additional

with the organism itself, it was not surprising that

s be useful.

Because the ELISA detects circulating antibodies to M paratuberculosis and bacterial culture of feces detects the organism itself, it was not surprising that

earners focused at reducing risks of transmission of the organ-

cows in these 8 herds (8/121, 1/65, 14/56, 5/98, 2/40, 2/32, 14/48, and 17/58 cows), even though results of bacterial culture of fe-

infection status is also the key to the development of the VJDHSP in the United States, and results of the present study provide information about the validity of the current VJDHSP methods for identifying paratuberculosis in dairy cattle herds.

In this study, information from 64 dairy herds in 5 states was compiled. All laboratories used the same commercially available ELISA kit for detection of anti-

molar evidence of paratuberculosis, the probability of misclassification (ie, identification of herd as having paratuberculosis) ranged from 89 to

of fecal samples for

tatuberculosis status. Sensitivity of bacterial culture

disease in the animal,6 with sensitivity estimated to be

interpretation of herd test data is complicated by

state, rather than as a single value for all herds. Additional

results of the 2 tests did not always agree for individual
cattle. Interpretation of herd test data is complicated by the lack of a true gold standard for determining herd paratuberculosis status. Sensitivity of bacterial culture of fecal samples for M paratuberculosis in subclinically infected cattle has been estimated to be only 40 to

sitivity may be even lower (33%). On the other hand, specificity of bacterial culture is considered to be 100%. Thus, bacterial culture is the gold standard used in the current VJDHSP and in the present study.

Because of concerns about animal health and potential public health aspects of paratuberculosis, there is a need to accurately identify cattle herds infect-

or are not infected with

Table 2—Herd sensitivity of using a commercial ELISA alone, without follow-up confirmatory bacterial culture of fecal samples from cows with positive ELISA results, for detection of paratuberculosis in dairy cattle herds

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of herds</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>All cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of cows with positive bacterial culture results</td>
<td>0</td>
<td>8</td>
<td>89% (6%)</td>
<td>94% (5%)</td>
<td>97% (3%)</td>
</tr>
<tr>
<td>0.1 to 4.9</td>
<td>22</td>
<td>70% (7%)</td>
<td>76% (7%)</td>
<td>79% (7%)</td>
<td>91% (6%)</td>
</tr>
<tr>
<td>5 to 9.9</td>
<td>11</td>
<td>84% (9%)</td>
<td>90% (9%)</td>
<td>91% (9%)</td>
<td>91% (9%)</td>
</tr>
<tr>
<td>≥10</td>
<td>23</td>
<td>93% (5%)</td>
<td>97% (2%)</td>
<td>98% (1%)</td>
<td>100% (0%)</td>
</tr>
<tr>
<td>All herds with infected cows</td>
<td>56</td>
<td>82% (6%)</td>
<td>87% (4%)</td>
<td>89% (4%)</td>
<td>95% (3%)</td>
</tr>
<tr>
<td>Model R²</td>
<td>0.12</td>
<td>0.11</td>
<td>0.10</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

See Table 1 for key.
in dairy herds shed low numbers of organisms in their feces. Because of this, estimated test sensitivity used in development of the VJDHSP (25%) was lower than that previously reported in the literature (45%). 0.8, 0.0 Because of the low test sensitivity and imperfect test specificity, the probability of false-positive test results poses a substantial problem when multiple animals are tested from a single herd. With the currently approved ELISA, it is expected that > 25% of uninfected herds will have at least 1 false-positive test result if 30 cows are tested in each herd and that the percentage of herds with false-positive test results will increase as the number of cows tested increases. 0.7

One approach for dealing with this high probability of false-positive test results when using the ELISA to determine herd infection status is to create a category of indeterminate herd infection status and use additional testing to further define herd infection status. A second approach, used by the National Animal Health Monitoring System, involves incorporating secondary sources of information (eg, percentage of cows culled in the previous 12 months with clinical signs of paratuberculosis). This approach worked adequately for estimating national and regional prevalences of paratuberculosis but is unsatisfactory for determining individual herd infection status. A third approach, used in the model VJDHSP, requires confirmation of positive ELISA results with follow-up bacterial culture of fecal samples. Because of the presumed 100% specificity of bacterial culture, this method should eliminate the possibility of falsely identifying uninfected herds as infected. A fourth approach, representing a modification of the VJDHSP used by a few states, is to rely solely on results of the ELISA, without requiring bacterial culture for confirmation of positive ELISA results. The present study specifically examined results of using the latter 2 methods. A further VJDHSP criterion that only those cows in their second or greater lactation be tested could not be examined in the present study because of a lack of information on cow ages for all study herds.

Herd sensitivity of various test strategies can be estimated theoretically with standard software. For instance, if we assume that sensitivity of the ELISA is 25%, sensitivity of bacterial culture of fecal samples is 40%, and specificity of the combination of ELISA and bacterial culture is 100%, then for a herd in which 10% of the cows had positive bacterial culture results, the true prevalence of infection would be 25%, and the theoretical probabilities of identifying the herd as infected (ie, detecting at least 1 cow with positive ELISA and bacterial culture results) would be 0.86, 0.93, and 0.97 when collecting samples from 30, 40, or 50 cows. If 5% of the cows in the herd had positive bacterial culture results, the true prevalence of infection would be 12.5%, and the theoretical probabilities of identifying the herd as infected would be 0.61, 0.72, and 0.80 when collecting samples from 30, 40, or 50 cows. If 1% of the cows in the herd had positive bacterial culture results, the true prevalence of infection would be 2.5%, and the theoretical probabilities of identifying the herd as infected would be 0.15, 0.19, and 0.24 when collecting samples from 30, 40, or 50 cows. These estimates are generally consistent with those generated in the present study, providing assurance that assumed test sensitivities and specificities were reasonably correct. This also points out an important problem in the VJDHSP associated with testing herds with a low prevalence of infection. In the present study, even if all adult cows in the herd were tested, only 73% of the herds in which bacterial culture results were positive for 0.1 to 4.9% of the cows would be identified as being infected if the VJDHSP protocol were followed (screening all cows with the ELISA and following up positive ELISA results with bacterial culture of feces). This must be considered when state regulatory officials, veterinarians, and producers consider test results at the herd level.

In summary, in the present study, herd sensitivity for detecting M paratuberculosis infection, using methods advocated in the VJDHSP, depended on percentage of cows with positive bacterial culture results, percentage of cows with positive ELISA results, and sample size. In particular, identifying a herd as infected was difficult for herds with a low prevalence of paratuberculosis, even when all cows in the herd were tested. Unfortunately, the distribution of within-herd prevalence of paratuberculosis among US dairy herds is not currently known.

References