Evaluation of performance of bacterial culture of feces and serum ELISA across stages of Johne's disease in cattle using a Bayesian latent class model

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ABSTRACT

The objective of this study was to evaluate the performance of bacterial culture of feces and serum ELISA to correctly identify cows with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) at heavy, light, and non-fecal-shedding levels. A total of 29,785 parallel test results from bacterial culture of feces and serum ELISA were collected from 17 dairy herds in Minnesota, Pennsylvania, and Colorado. Samples were obtained from adult cows from dairy herds enrolled for up to 10 yr in the National Johne's Disease Demonstration Herd Project. A Bayesian latent class model was fitted to estimate the probabilities that bacterial culture of feces (using 72-h sedimentation or 30-min centrifugation methods) and serum ELISA results correctly identified cows as high positive, low positive, or negative given that cows were heavy, light, and non-shedders, respectively. The model assumed that no gold standard test was available and conditional independency existed between diagnostic tests. The estimated conditional probabilities that bacterial culture of feces correctly identified heavy shedders, light shedders, and non-shedders were 70.9, 32.0, and 98.5%, respectively. The same values for the serum ELISA were 60.6, 18.7, and 99.5%, respectively. Differences in diagnostic test performance were observed among states. These results improve the interpretation of results from bacterial culture of feces and serum ELISA for detection of MAP and MAP antibody (respectively), which can support on-farm infection control decisions and can be used to evaluate disease-testing strategies, taking into account the accuracy of these tests.

Key words: paratuberculosis, fecal shedding, diagnostic test, dairy cow

INTRODUCTION

Johne's disease (JD) is a chronic infectious disease in cattle caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). In 2007, The USDA Animal and Plant Health Inspection Service (APHIS) estimated that at least 68% of US dairy farms are infected with MAP, based on testing of environmental fecal samples (USDA-APHIS, 2008). *Mycobacterium avium* ssp. *paratuberculosis* infection causes economic losses directly associated with premature culling as well as reduced milk production (Raizman et al., 2007). Management practices to control JD are focused not only on reducing the transmission of MAP from infected, shedding cows to uninfected young calves, but also on culling heavy-shedding cows as soon as they are detected (Raizman et al., 2007).

The disease has a slowly progressing detrimental effect on cow health and production. The progression of the disease can be categorized into multiple stages (Whitlock et al., 2000; Collins, 2002; Nielsen and Toft, 2008); however, based on their potential of transmitting MAP infection to susceptible cattle, a population can be categorized into 3 groups (Crossley et al., 2005):

- (1) Non-shedding cows. Although most animals in this group may not be infected with MAP, a proportion of them could be infected and in the latent stage of the disease. Currently, there is no practical way to differentiate latent-infected cows from noninfected cows (Whitlock et al., 2000).
- (2) Light-shedding cows. Nearly all light fecal shedders are infected with MAP and, in general, shed a low concentration of MAP into the environment that can be occasionally detected using diagnostic tests. A small proportion of these cows may transiently shed MAP because of an environment highly contaminated with MAP that passes through the digestive system of the cows (Sweeney et al., 1992). Light-shedding cows have

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a lower risk of transmitting disease to susceptible cattle compared with heavy shedders.

- (3) Heavy-shedding cows. This group represents infected cows in advanced stages of the infection. They shed large amounts of MAP in feces into the environment and are likely to be detected using diagnostic tests. These cattle represent the highest risk of disease transmission and their identification and culling is important for disease control (Lu et al., 2008).

Understanding and quantifying the MAP fecal shedding level of infected cattle in herds helps producers and veterinarians elaborate breeding lists, prioritize culling decisions on the most infectious cattle (heavy shedders) and implement specific management practices to reduce transmission of MAP infection within the herd. Categorizing infected cattle by their potential risk of disease transmission using available diagnostic tests would also assist in understanding the dynamics of disease transmission, implementing accurate disease transmission models, and evaluating the impact and effectiveness of MAP disease control or eradication programs (Lu et al. 2008).

The most commonly used diagnostic tests for MAP infection are bacterial culture of feces and serum ELISA. Several studies have estimated the sensitivity and specificity of these diagnostic tests, using bacterial cultures of feces to confirm the infection or infectious status of the cattle, repeated testing in some cases, or by using samples from confirmed negative herds (Whitlock et al., 2000; Collins et al., 2005; Wells et al., 2006). In contrast, only a few studies have estimated the performance of serum ELISA to detect cows with either low or high positive results of fecal bacterial culture (Wells et al., 2006; Donat et al., 2014). Although a Bayesian approach was previously used to estimate the performance of diagnostic tests for MAP infection in absence of a gold standard (Wells et al., 2006; Norton et al., 2010), this approach has been previously used to estimate the performance of binary (positive or negative) test interpretation, with a target condition categorized based on infection or infectious status. The objective of this study was to estimate the accuracy of the bacterial culture of feces and serum ELISA to identify MAP heavy-, light-, and non-shedding cattle using a Bayesian approach without a gold standard test.

MATERIALS AND METHODS

Mycobacterium avium ssp. *paratuberculosis* infection diagnostic test results from the National Johne's Disease Demonstration Herd Program (JDDHP) were

obtained from the USDA-APHIS: Veterinary Services (Fort Collins, CO). Herds were selected using the following criteria: (1) only herds with whole adult herd test results available; (2) only herds with test results from both bacterial culture of feces on Herrold's egg yolk medium (HEYM) using either 72-h sedimentation or 30-min centrifugation method (Eamens et al., 2000) and serum ELISA (Idexx Laboratories Inc., Westbrook, ME); (3) only herds in which fecal samples for bacterial culture of feces and blood samples for serum ELISA were collected from cattle on the same day; and (4) only herds that did not report implementation of vaccination against JD during the study period.

Diagnostic test results from 17 states and 66 herds were obtained, and 8 herds from Minnesota (MN), 7 herds from Pennsylvania (PA), and 2 herds from Colorado (CO) fulfilled the inclusion criteria. In those herds, samples were annually collected from 2000 to 2009 in MN, from 2002 to 2010 in PA, and from 2003 to 2004 in CO, depending on the date of herd enrollment in the JDDHP. In almost all herds, samples were collected annually in approximately the same month every year to allow 12 mo between sample collections within herds; however, in 2 herds in Minnesota, sample collection was based on stage of lactation of the cows from 2000 to 2004, and changed to annual testing in approximately the same month every year for the subsequent years of the study. Cows were at least 24 mo of age when samples were collected. Adult cow herd sizes varied from approximately 50 to 400, 50 to 800, and 300 to 400 adult cows in MN, PA, and CO, respectively (Table 1). Holstein was the predominant breed in all herds.

After collection, serum and fecal samples from MN and PA were sent for testing to the Minnesota Veterinary Diagnostic Laboratory (St. Paul) and Pennsylvania Animal Diagnostic Laboratory System (University Park), respectively. Serum samples from CO were sent to the Colorado State University Veterinary Diagnostic Laboratories (Fort Collins), whereas fecal samples from CO were sent to Wisconsin Veterinary Diagnostic Laboratory (Madison). Fecal samples were submitted to the respective laboratories to perform bacterial culture of feces using HEYM. Fecal samples from MN and CO were processed using the 72-h sedimentation procedure before culture, and fecal samples from PA were processed using the 30-min centrifugation procedure (Eamens et al., 2000). Serum samples were tested using serum ELISA (Idexx Laboratories Inc.) in all 3 laboratories. During the study period, 29,785 paired test results from bacterial culture of feces and serum ELISA were collected from MN ($n = 12,970$), PA ($n = 15,680$), and CO ($n = 1,135$, Table 2). All these

Table 1. Dairy herd characteristics, apparent prevalence of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) bacterial culture of feces and Johne's disease serum ELISA, and true prevalence of MAP fecal shedding cows in 17 commercial dairy herds (8 in Minnesota, 2 in Colorado, and 7 in Pennsylvania)

Herd	State	Cows tested, mean ¹	Prevalence bacterial culture of feces (%) ²	Prevalence serum ELISA (%) ²	True prevalence (%) ³	
					All shedders	Heavy
1	Minnesota	230	14.7	28.5	30.0	4.8
2	Minnesota	41	38.3	8.3	20.4	4.4
3	Minnesota	246	3.6	3.2	3.1	1.6
4	Minnesota	316	6.7	8.8	6.6	0.9
5	Minnesota	37	15.9	18.2	12.1	5.4
6	Minnesota	285	15.9	6.5	10.1	3.0
7	Minnesota	356	21.8	9.2	16.0	2.8
8	Minnesota	228	13.8	11.4	13.3	2.5
9	Pennsylvania	596	4.7	2.6	10.9	1.6
10	Pennsylvania	199	7.9	4.5	6.9	1.9
11	Pennsylvania	338	6.3	2.7	6.0	1.2
12	Pennsylvania	422	5.8	8.3	7.7	2.4
13	Pennsylvania	131	1.9	1.0	1.9	0.4
14	Pennsylvania	66	9.8	6.6	8.5	1.5
15	Pennsylvania	375	3.4	3.8	1.6	0.7
16	Colorado	408	3.9	2.9	6.1	0.4
17	Colorado	355	5.3	3.9	6.4	0.9

¹Mean adult cows (age >24 mo) tested per year.²Annual apparent prevalence at the beginning of the study.³Bayesian estimation of the true prevalence of *Mycobacterium avium* ssp. *paratuberculosis* fecal shedding (all shedders) and heavy fecal-shedding (heavy) cows for the study period.

laboratories passed annual USDA-APHIS-Veterinary Services proficiency tests administered by the National Veterinary Services Laboratories (Ames, IA).

This study used the following terminology to differentiate test results from the true fecal shedding status of the cattle: diagnostic test results were classified as high, low, or negative, and true fecal shedding status of the cattle were classified as heavy, light, or non-shedder. A diagnostic test correctly identified heavy, light, and non-shedders if the diagnostic test result was high, low, and negative, respectively.

Table 2. Distribution of the combined *Mycobacterium avium* ssp. *paratuberculosis* test results from bacterial culture of feces and serum ELISA in 17 commercial dairy herds (8 in Minnesota, 2 in Colorado, and 7 in Pennsylvania)

Bacterial culture ¹	Serum ELISA ²	Number
Negative	Negative	27,341
Low	Negative	1,004
High	Negative	119
Negative	Low	490
Low	Low	172
High	Low	108
Negative	High	111
Low	High	192
High	High	248
Total		29,785

¹Negative bacterial culture of feces = 0 colonies/slant, Low = >0 and <50 colonies/slant, High = ≥50 colonies/slant.²Negative ELISA = sample to positive ratio <0.25, Low = sample to positive ratio ≥0.25 and <1.0, High = sample to positive ratio ≥1.0.

Serum ELISA results were classified using different cutoff points for the sample-to-positive ratio (**S/P**), where negative samples had an S/P <0.25 and positive samples had an S/P ≥0.25. Positive samples were further classified as low positive when $0.25 \leq \text{S/P} < 1.0$ and high positive when the S/P was ≥1.0 (Collins, 2002). Similarly, test results from bacterial culture of feces were classified as negative when no MAP was detected and as positive when >0 colonies of MAP were observed. Positive samples were also divided into low positive when the bacterial culture of feces results indicated an average of >0 and <50 MAP colonies per tube and high positive when the bacterial culture of feces result was an average of ≥50 MAP colonies per tube (Crossley et al., 2005).

Analysis

A Bayesian latent-class model was developed using Markov Chain Monte Carlo (**MCMC**) methods to estimate the conditional probability that diagnostic test results correctly or incorrectly identified a true heavy, light, or non-fecal shedding cow. A latent-class model was used because the true MAP fecal shedding levels of the cows (latent-class variables) are unobserved conditions imperfectly measured by the diagnostic tests (Congdon, 2006). The model builds on the model initially described by Hui and Walter (1980) and Johnson et al. (2001) for one test with a binomial outcome, one population, and no gold standard.

Given the 3 possible test results for bacterial culture of feces ($\mathbf{T}_x^C$) where x represented the test results: high ($\mathbf{T}_H^C$), low ($\mathbf{T}_L^C$) and negative ($\mathbf{T}_N^C$), the 3 possible test results for serum ELISA ($\mathbf{T}_y^E$), where y represented the test results: high ($\mathbf{T}_H^E$), low ($\mathbf{T}_L^E$), and negative ($\mathbf{T}_N^E$), and the 3 possible true fecal shedding status ($\mathbf{S}_j$) where j represents the true fecal shedding status: heavy ($\mathbf{S}_H$), light ($\mathbf{S}_L$), and non-shedder ($\mathbf{S}_N$), the conditional probabilities of the bacterial culture of feces test result given the true fecal shedding status of the cows was $\mathbf{T}_x^C|\mathbf{S}_j$. Similarly, the conditional probabilities of the serum ELISA test result given the true fecal shedding status of the cows was $\mathbf{T}_y^E|\mathbf{S}_j$.

The Bayesian latent-class model was built assuming conditional independency between diagnostic tests; that is, test results are independent but conditional on the true fecal shedding status. This model also assumed that there was no available gold standard test and the test performances were independent of herds and cows. Herds were considered as independent populations in the model. Cows in the k herd, where $k = 1$ to 17 herds in this study, had 9 possible combinations of bacterial culture of feces and serum ELISA test results ($\mathbf{T}_x^C$ and $\mathbf{T}_y^E$, respectively) and the number of cows in each possible combination of diagnostic test results for each herd was given by the vector $\mathbf{y}_{xyk} = (y_{HHk}, y_{HLk}, y_{HNk}, y_{LHk}, y_{LLk}, y_{LNk}, y_{NHk}, y_{NLk}, y_{NNk})$. The sum of all the elements of the vector $\mathbf{y}_{xyk}$ was equal to the size of the population in the herd k (n_k). Similarly, the vector $\mathbf{p}_{xyk}$ represented the proportion of cows in the herd k in either of the 9 possible combinations of bacterial culture of feces and serum ELISA test results ($\mathbf{T}_x^C$ and $\mathbf{T}_y^E$, respectively) and was described as $\mathbf{p}_{xyk} = (p_{HHk}, p_{HLk}, p_{HNk}, p_{LHk}, p_{LLk}, p_{LNk}, p_{NHk}, p_{NLk}, p_{NNk})$ and the sum of all the elements of the vector $\mathbf{p}_{xyk}$ added to 1. The true prevalences of fecal shedder was given by π_{jk} , where j represented heavy ($\mathbf{H}$), light ($\mathbf{L}$), and non- ($\mathbf{N}$) shedders and k represented the k th herd.

Therefore, assuming that each test-day and cow represented an independent observation in the k th herd, the probability of the joint bacterial culture of feces and serum ELISA diagnostic test results $\mathbf{y}_{xyk}$ had a multinomial distribution as follows:

$$\mathbf{y}_{xyk} \sim \text{Multinomial}(n_k, \mathbf{p}_{xyk}),$$

and the probability of a combined bacterial culture and serum ELISA test result in the k herd was given by

$$\begin{aligned} \mathbf{p}_{xyk} = & \pi_{Hk} (\mathbf{T}_x^C|\mathbf{S}_H) (\mathbf{T}_y^E|\mathbf{S}_H) + \pi_{Lk} (\mathbf{T}_x^C|\mathbf{S}_L) (\mathbf{T}_y^E|\mathbf{S}_L) \\ & + \pi_{Nk} (\mathbf{T}_x^C|\mathbf{S}_N) (\mathbf{T}_y^E|\mathbf{S}_N). \end{aligned}$$

Model outcomes were the conditional probability of test results given the true fecal shedding status of the cattle for bacterial culture of feces and serum ELISA, and the true prevalence of heavy, light, and non-shedders in the k herd, π_{Hk} , π_{Lk} , and π_{Nk} , respectively. The model estimated 9 conditional probabilities for each test and 3 fecal shedding true prevalences for each herd; therefore, the model estimated 69 parameters with 40 degrees of freedom. The analysis was also stratified by state to evaluate differences on the test performance by state.

Using Bayes' theorem, MCMC was used to estimate the posterior distribution of the predictive values of heavy, light, and non-shedders for the bacterial culture of feces and serum ELISA in each k herd. The predicted values of the heavy fecal shedders corresponded to the conditional probability of heavy fecal shedder given that the bacterial culture of feces or the serum ELISA result was high, $(\mathbf{S}_H|\mathbf{T}_H^C)$ and $(\mathbf{S}_H|\mathbf{T}_H^E)$, respectively. Similarly the predicted value of the light fecal shedder corresponded to the conditional probability of light fecal shedder given that the bacterial culture of feces or the serum ELISA result was low, $(\mathbf{S}_L|\mathbf{T}_L^C)$ and $(\mathbf{S}_L|\mathbf{T}_L^E)$, respectively. Finally, the predicted value of the non-shedder corresponded to the conditional probability of non-shedder given that the bacterial culture of feces or the serum ELISA result was negative, $(\mathbf{S}_N|\mathbf{T}_N^C)$ and $(\mathbf{S}_N|\mathbf{T}_N^E)$, respectively.

Informative prior distributions for the conditional probabilities of diagnostic test results ($\mathbf{T}$) high, low, or negative ($\mathbf{T}_H$, $\mathbf{T}_L$, and $\mathbf{T}_N$, respectively) given the true fecal shedding status ($\mathbf{S}$) of heavy, light, or non-shedding cows ($\mathbf{S}_H$, $\mathbf{S}_L$, $\mathbf{S}_N$, respectively), were modeled using a Dirichlet distribution parameterized by the vector $\boldsymbol{\alpha}_x^C$ for bacterial culture of feces ($\boldsymbol{\alpha}_H^C$, $\boldsymbol{\alpha}_L^C$, and $\boldsymbol{\alpha}_N^C$) and the vectors $\boldsymbol{\alpha}_y^E$ for serum ELISA ($\boldsymbol{\alpha}_H^E$, $\boldsymbol{\alpha}_L^E$, and $\boldsymbol{\alpha}_N^E$). The values of the prior conditional probabilities of diagnostic test results for both diagnostic tests were assigned by consensus between 3 experts [Scott Wells, Ian Gardner (University of Prince Edward Island, Charlottetown, Canada) and Elizabeth Patton (Wisconsin Department of Agriculture, Madison)]; Table 3]:

$$\mathbf{P}^C(\mathbf{T}_x^C|\mathbf{S}_j) \sim \text{Dirichlet}(\boldsymbol{\alpha}_x^C)$$

$$\mathbf{P}^E(\mathbf{T}_y^E|\mathbf{S}_j) \sim \text{Dirichlet}(\boldsymbol{\alpha}_y^E).$$

Because prior information of the herd true prevalences (π_{ijk}) of heavy (π_{Hk}), light (π_{Lk}), and non-shedders (π_{Nk}) were not available for all k herds, they were included in the model using noninformative Dirichlet prior distributions:

$$\pi_{jk} \sim \text{Dirichlet}(\pi_{Hk}, \pi_{Lk}, \pi_{Nk}).$$

Table 3. Mean and 95% credible interval (95% CrI) of the posterior conditional probability of *Mycobacterium avium* ssp. *paratuberculosis* bacterial culture of feces and serum ELISA test results given the true status of the cattle, using informative and alternative priors for the conditional probabilities of diagnostic test results

Test and results ¹	Fecal shedding true status	Informative prior probability (%)	Conditional probability (%)		Alternative prior probability ² (%)	Conditional probability ² (%)	
			Mean	95% CrI		Mean	95% CrI
Bacterial culture of feces							
Negative	Non	95	98.5	98.1–98.9	90	98.3	97.9–98.7
Low	Non	4	1.4	1.0–1.7	8	1.4	1.1–1.8
High	Non	1	0.2	0.04–0.3	2	0.2	0.1–0.3
Negative	Light	45	66.4	59.7–72.6	40	63.2	55.3–70.2
Low	Light	45	32.0	26.0–38.3	50	35.0	28.1–42.7
High	Light	10	1.6	0.8–2.7	10	1.8	1.0–2.9
Negative	Heavy	3	2.4	0.5–5.5	2	1.6	0.2–4.2
Low	Heavy	12	26.7	18.3–33.7	8	24.1	13.2–32.5
High	Heavy	85	70.9	63.6–79.6	90	74.4	65.6–85.7
Serum ELISA							
Negative	Non	98	99.5	99.3–99.7	90	99.3	99.1–99.5
Low	Non	1.5	0.5	0.3–0.7	8	0.6	0.4–0.8
High	Non	0.5	0.01	<0.001–0.05	2	0.04	0.01–0.1
Negative	Light	80	74.4	68.7–79.6	85	73.4	67.4–79.0
Low	Light	15	18.7	15.4–22.3	13	18.9	15.6–22.4
High	Light	5	6.9	4.4–9.7	2	7.7	4.8–11.3
Negative	Heavy	15	13.5	8.0–19.7	8	7.6	3.4–13.2
Low	Heavy	35	25.9	22.0–30.0	44	29.2	25.2–33.5
High	Heavy	50	60.6	55.0–66.4	48	63.2	57.6–68.3

¹Bacterial culture of feces: negative = 0 colonies/slant, Low >0 and <50 colonies/slant, High ≥ 50 colonies/slant. Serum ELISA: negative = sample to positive ratio <0.25, Low = sample to positive ratio ≥0.25 and <1.0, High = sample to positive ratio ≥1.0.

²Alternative informative priors and corresponding posterior conditional probabilities.

Models were implemented using OpenBugs (Thomas et al., 2006) and run using 3 parallel chains in the MCMC with sparse initial values and 30,000 iterations per chain. The first 500 iterations were discarded in each chain as a burn-in period. Thinning of the iterations was performed to eliminate autocorrelation. Posterior distributions of the diagnostic test conditional probabilities were summarized using the mean and the 95% credible interval (**CrI**). Convergence of the model was visually evaluated using trace plots and Gelman-Rubin diagnostic plots (Brooks and Gelman, 1998).

The sum of the conditional probabilities that the bacterial culture of feces (I^C) or serum ELISA (I^E) correctly identified a heavy, light, or non-shedder was used to obtain an index that summarized the performance of each test (I^C and I^E):

$$I^C = P^C(T^C_H|S_H) + P^C(T^C_L|S_L) + P^C(T^C_N|S_N),$$

$$I^E = P^E(T^E_H|S_H) + P^E(T^E_L|S_L) + P^E(T^E_N|S_N).$$

Percentage of changes of these indexes (PC^{IC} and PC^{IE}) was used to evaluate the influential effect of each of the k th herds on the estimated parameters for test performance, as an indicator of the sensitivity of the estimated parameter for the diagnostic test performance to the herds under study. Therefore, PC^C and PC^E equals 0 indicate no effect of the herd k th on the indexes. Percentage of changes of these indexes was calculated using

$$PC^{IC}_k = [(I^C \text{ without the } k\text{th herd} / I^C \text{ with all herds}) - 1] \times 100,$$

$$PC^{IE}_k = [(I^E \text{ without the } k\text{th herd} / I^E \text{ with all herds}) - 1] \times 100.$$

A new set of alternative priors was used to evaluate the effect of the informative priors for the conditional probabilities of diagnostic test results for both diagnostic tests on the posteriors estimated conditional probabilities. Alternative priors were included in the same Bayesian latent-class model and their values were chosen by consensus between 2 of the authors (LE and SW) to evaluate the influence of the priors on the posterior conditional probabilities distributions of the test performances. In this analysis, alternative priors selected were up to 10 percentage units from the informative priors to evaluate the influence of the priors on movement of the posterior conditional probabilities in the direction of the priors.

RESULTS

A total of 29,785 samples were collected from 13,265 cattle, including 8,130 tested more than once (range 2–8). The apparent within-herd prevalence of MAP by bacterial culture of feces and serum ELISA in the first year of the study ranged from 1.9 to 38.3% and from 1.0 to 28.5%, respectively (Table 1).

Heavy Fecal Shedders

The posterior probabilities that bacterial culture of feces and serum ELISA correctly identified a heavy fecal shedding cow as high positive were 70.9% (CrI = 63.6 to 79.6) and 60.6% (55.0 to 66.4), respectively (Table 3). The proportion of heavy-shedding cows that were incorrectly classified as low was similar between bacterial culture of feces and serum ELISA; however, the proportion of heavy shedders that were classified as negative was almost 6 times higher for serum ELISA than for bacterial culture of feces.

The stratified analysis indicated differences in the performance of bacterial culture of feces by state (Table 4). Overall, bacterial culture of feces and serum ELISA performed similarly in PA and CO. The conditional probability of correctly identifying heavy shedders as high using bacterial culture of feces was approximately 23% lower in MN compared with these values in CO and PA. In MN, most of the heavy-shedding cows that were not correctly classified as high by bacterial culture of feces were instead classified as low shedders. The false negative rate of bacterial culture of feces for heavy-shedding cows was low and similar among the 3 states (Table 4).

In contrast, the serum ELISA in MN showed a higher probability of correctly identifying heavy shedders as high compared with test performance in PA and CO. The probabilities of the serum ELISA incorrectly classifying heavy shedders as low or negative was similar among states (Table 4).

Light Fecal Shedders

The estimated probabilities that light fecal shedders would be correctly identified as low shedders by the bacterial culture of feces and serum ELISA were 32.0% (26.0 to 38.3) and 18.7% (15.4 to 22.3), respectively. Most of the light shedders were false negative to the bacterial culture of feces and serum ELISA, and a small proportion of the light shedders were identified as high when using these diagnostic tests (Table 3).

The conditional probability of bacterial culture of feces to correctly identify light shedders as low was

lower in MN compared with PA and CO (Table 4); however, light shedders were more likely to be classified as false negative and less likely to be classified as false heavy shedders in MN than in CO and PA (Table 4). Similarly, the conditional probability of serum ELISA to correctly identify light shedders as low was approximately twice the values observed in PA (Table 4).

Non-Shedders

The estimated probability that bacterial culture of feces and serum ELISA correctly identified a non-shedder as negative (specificity) was 98.5% (98.1 to 98.9) and 99.5% (99.3 to 99.7), respectively (Table 3). Most of the false-positive test results were low positive and only a small proportion of the false positives were high positive in either diagnostic test.

The specificity of bacterial culture of feces and serum ELISA was similar across states, as was the conditional probability of high test results in non-shedders. The probability of the bacterial culture of feces to incorrectly classify a non-shedder as low was approximately 3 times higher in MN than in PA (Table 4).

True Prevalence and Predictive Values

The true prevalence of fecal-shedding cows throughout the study period was generally higher in MN herds

compared with PA and CO herds (Table 1). The estimated within-herd true prevalence (95% CrI) of heavy fecal shedders for the study period varied from 0.4% (0.1 to 1.1) to 5.4% (2.9 to 8.5). Likewise, the estimated within-herd true prevalence of light fecal shedders for the study period varied from 0.9% (0.1 to 2.1) to 25.2% (20.5 to 30.6). The ratio of the true prevalence of fecal shedders to true prevalence of heavy fecal shedder varied from 1.9 to 16.0 (Table 1).

As expected, positive predictive values of bacterial culture of feces and serum ELISA increased with the true prevalence of heavy, light, and non-shedders within the ranges of true prevalences observed in the population of herds and period under study. The estimated predicted value for heavy shedding using bacterial culture of feces was high and varied from 56.2% (6.1 to 88.4) to 92.1% (83.5 to 97.1). The predicted value for heavy fecal shedding given that the serum ELISA was high varied by herd from 37.3% (2.1 to 78.0) to 89.8% (74.6 to 99.0). Similarly, the estimated predictive values for light fecal shedding varied by herd from 14.0% (0.7 to 36.6) to 81.2% (74.7 to 87.1) for bacterial culture of feces and from 28.5% (2.9 to 58.3) to 78.2% (72.0 to 83.7) for serum ELISA. The negative predictive values for non-shedding varied by herd from 78.4% (72.0 to 83.6) to 99.6% (98.7 to 100) for negative bacterial culture of feces and from 76.1% (69.9 to 81.2) to 99.4% (98.5 to 99.9) for negative serum ELISA (Figure 1).

Table 4. Mean and 95% credible interval (95% CrI) of the distribution of the posterior conditional probability of *Mycobacterium avium* ssp. *paratuberculosis* bacterial culture of feces and Johne's disease serum ELISA test results given the true status of the cattle, in 8 dairy herds in Minnesota, 7 in Pennsylvania, and 2 in Colorado

Diagnostic test and results ¹	Fecal shedding true status	Minnesota		Colorado		Pennsylvania	
		Mean	95% CrI	Mean	95% CrI	Mean	95% CrI
Bacterial culture of feces							
Negative	Non	97.7	96.8–98.5	98.6	97.4–99.5	99.2	98.7–99.6
Low	Non	2.3	1.5–3.2	1.3	0.4–2.4	0.7	0.3–1.1
High	Non	0.01	<0.01–0.02	0.1	<0.01–0.4	0.1	<0.01–0.3
Negative	Light	68.2	61.9–73.9	46.3	36.8–56.0	49.8	40.7–58.6
Low	Light	29.7	24.3–35.7	46.2	37.1–55.5	43.4	35.4–51.8
High	Light	2.1	1.1–3.3	7.5	3.8–12.5	6.8	4.0–10.2
Negative	Heavy	2.7	0.6–6.2	3.0	0.7–7.2	4.6	1.0–10.1
Low	Heavy	37.1	30.6–43.2	13.6	7.3–21.6	11.7	6.8–17.3
High	Heavy	60.2	53.6–67.3	83.4	75.0–90.3	83.7	76.1–90.2
Serum ELISA							
Negative	Non	99.4	98.9–99.8	98.9	97.9–99.6	99.3	99.1–99.5
Low	Non	0.5	0.1–0.9	0.8	0.2–1.6	0.7	0.4–0.8
High	Non	0.1	<0.01–0.2	0.3	<0.01–0.9	0.01	<0.01–0.1
Negative	Light	69.4	62.3–75.8	74.4	66.2–82.1	83.3	78.7–87.5
Low	Light	24.2	19.2–29.8	14.5	8.9–21.3	11.4	8.2–15.0
High	Light	6.4	4.0–9.4	11.1	5.6–17.3	5.3	3.0–7.8
Negative	Heavy	9.6	5.8–13.8	14.6	8.9–21.3	14.4	8.7–20.9
Low	Heavy	22.0	17.5–26.9	35.7	26.8–45.1	32.3	26.7–38.3
High	Heavy	68.4	62.9–73.9	49.7	40.0–59.3	53.3	46.6–59.9

¹Bacterial culture of feces negative = 0 colonies/slant, Low >0 and <50 colonies/slant, High ≥50 colonies/slant. Serum ELISA negative = sample to positive ratio <0.25, Low = sample to positive ratio ≥0.25 and <1.0, High = sample to positive ratio ≥1.0.

Figure 1. Mean (solid line) and 95% credible interval (dotted lines) of *Mycobacterium avium* ssp. *paratuberculosis* bacterial culture of feces (a, c, e) and Johne's disease serum ELISA (b, d, f) predictive values of high (a, b), low (c, d) and negative (e, f) test results by true prevalence of heavy, light, and non-shedding, in cattle in 8 dairy herds in Minnesota, 7 in Pennsylvania, and 2 in Colorado.

Analysis of Test Performance

The analysis of the effect of herd on test performance was performed using the percentage change of the sum of the probabilities that either bacterial culture of feces or serum ELISA correctly identified a heavy shedder as high, light shedder as low, and non-shedder as negative. The absolute value of the performance change of bacterial culture of feces varied from 0.2 to 15.3%. Similarly, the absolute value of the performance change of the serum ELISA varied from 0 to 6.0%.

One herd from MN had the most influence on the estimated performance of bacterial culture of feces and serum ELISA (Figure 2). When this herd was excluded from the analysis, the probabilities of bacterial culture of feces correctly identifying a heavy shedder as high, light shedder as low, and non-shedders as negative were 84.4% (76.7 to 90.7), 50.4% (44.0 to 56.9), and 99.2% (98.8 to 99.6), respectively; while the same probabilities for serum ELISA were 55.3% (49.7 to 61.1), 13.5% (11.2 to 16.1), and 99.2% (99.0 to 99.4), respectively.

The analysis using alternative priors showed the magnitude of the absolute changes on the posterior conditional probabilities was lower than the change in the priors. The highest absolute change of the posterior conditional probabilities of the test performance when

compared informative with alternative priors was observed in the group of heavy fecal shedders.

DISCUSSION

To our knowledge, this is the first study to evaluate the accuracy of bacterial culture of feces and serum ELISA using a 3-level multinomial test result: negative, low, and high to correctly classify non-, light, and heavy shedders, respectively. This study used a Bayesian latent-class analysis approach that allowed obtaining posterior distributions of the diagnostic test performance given the test results (negative, low positive, and high positive) and the prior information of the diagnostic test performances and the prevalence of the fecal shedders. Unlike other published information about diagnostic test performance (Whitlock et al., 2000; Collins et al., 2005; Donat et al., 2014), this approach does not assume a gold standard or a reference test; however, in this analysis we used 2 conditionally independent test and prior estimations to provide information about the true fecal shedding status of the cows (non, light, and heavy shedder).

Bacterial culture of feces and serum ELISA are diagnostic tests that measure different biological phenomena in cattle; therefore, this study assumed that (1)

Figure 2. Sensitivity analysis of the herd effect on the percentage of change of the sum of the conditional probabilities that *Mycobacterium avium* ssp. *paratuberculosis* bacterial culture of feces (black) and Johne's disease serum ELISA (gray) correctly identify a heavy, light, or non-fecal shedder as high, low, and negative, respectively, in 17 Johne's disease demonstration herds.

both test results were conditionally independent, and (2) no other factors influenced test results other than the true fecal shedding status of the cattle (Gardner et al., 2000). This study also used the test threshold values recommended in the literature and commonly used to classify cattle based on the test results (Collins, 2002; Crossley et al., 2005).

Heavy- and light-shedding cows primarily represent infected cattle that actively shed MAP in feces because of the colonization of MAP into intestinal tissue (Stabel, 2000); however, a small proportion of light fecal shedders could be attributed to passive fecal shedding of MAP. These passive fecal shedders may test positive to bacterial culture of feces (likely to be low positive; Fecteau and Whitlock, 2010) and reduce the specificity of the tests.

Data collected in this study were treated as cross-sectional data; therefore, test results from the same cow and herds in different years were treated as independent observations. Similarly, estimated prevalences of heavy, light, and non-shedders were computed for the whole study period in each herd; however, these herds were under a disease control program and a decrease of the prevalence of fecal shedders throughout the time was expected.

Bacterial culture of feces correctly identified as positive (high or low) approximately 98% of the heavy-shedding cows; however, only approximately 34% of the light shedders were identified as positive (high or low) using bacterial culture of feces. Light shedders are likely shedding low levels of MAP that are sometimes undetectable when using bacterial culture of feces. Even though serum ELISA correctly identified most of the heavy fecal shedders as positive (approximately 86%), it was less accurate than bacterial culture of feces in this group of cows. Similar to results of bacterial culture of feces, most light fecal shedders were identified as negative by serum ELISA (approximately 74%). Despite the lower sensitivity of serum ELISA compared with bacterial culture of feces to identify MAP-positive cattle, as well as to identify their fecal shedding status (light or heavy), serum ELISA is a valuable diagnostic test (Nielsen and Toft, 2008), especially considering that it is a less expensive test and has shorter turnaround time than bacterial culture of feces.

Although most heavy shedders were correctly identified as high-positive by both diagnostic tests, the probability of a negative (false negative) test result for a heavy shedder was approximately 6 times higher for serum ELISA than for bacterial culture. In contrast, most light shedders had negative (false negative) bacterial culture and serum ELISA test results, indicating poor sensitivity of both diagnostic tests in this group of

cows. The estimated conditional probabilities of a positive (low or high) serum ELISA given a light or a heavy fecal shedder were previously estimated by Wells et al. (2006) to be 12.4 and 72.7%, respectively, and by Aly et al. (2012) to be 15 and 68.4%, respectively, using bacterial culture as a reference test. These values were lower than those estimated in this study (25.8 and 86.1%, respectively) for the same diagnostic test. However, our results were similar to those reported in another study that estimated a probability of positive serum ELISA between 15.2 to 24.3% in bacterial culture low-positive cows and between 70.6 to 100% in bacterial culture high-positive cows (Donat et al., 2014).

Our results estimated the specificity of the serum ELISA to be approximately 1 percentage unit higher than bacterial culture of feces. In our data, approximately 2% of test results were from serum ELISA-positive and bacterial culture-negative cows, and approximately 3.7% of test results corresponded to bacterial culture-positive and serum ELISA-negative cows. The estimated specificity is also influenced by the informative prior used. Experts set a prior specificity for bacterial culture of feces lower (95%) than that for serum ELISA (98%), because a small proportion of cows with positive bacterial culture results were expected to be false-positive as consequence of passive fecal shedding. Using another Bayesian approach, the specificity of the bacterial culture was previously estimated at 99.8% (Wells et al., 2006) and 98.0% (Alinovi et al., 2009) using the same culture technique.

The specificity of the serum ELISA in this study (99.5%, 95% CrI = 99.3 to 99.7), was higher than some of the values previously reported in the literature (Whitlock et al., 2000; Collins et al., 2005; Wells et al., 2006). Wells et al. (2006) previously reported a specificity of 94.9% using a similar Bayesian approach with 4 different tests to detect infection status. When samples were obtained from MAP infection-negative herds, Wells et al. (2006) and Collins et al. (2005) reported a specificity of 95.3 and 97.0%, respectively, for serum ELISA. Similarly, Whitlock et al. (2000) reported 98.9% using 3 serial bacterial cultures of feces to confirm negativity of the cows. The higher specificity for the serum ELISA compared with previously published information may be explained by the absence of a gold standard in this study or by the use of only 2 tests to inform the latent-class model. The higher specificity for the serum ELISA compared with previous publications could also be explained by an improvement in the specificity of the test over time. Additionally, these herds were under a disease control program; therefore, test-negative cows stayed in the herds longer and were tested more often than test-positive cows. The correlation between

samples within test-negative cows was not taken into account by the model and this might have increased the estimation of the specificity of the test.

Bacterial culture of feces performed better in correctly identifying light and heavy fecal shedders in PA and CO compared with MN. Light shedding cows were as likely to test positive (high or low) as test negative in PA and CO, and were more likely to test negative in MN. Differences in performance of bacterial culture of feces among states may be explained by differences in the MAP concentration procedure used before culture in the respective laboratories. Fecal samples sent to the MN Veterinary Diagnostic Laboratory were cultured using a 72-h sedimentation procedure for MAP concentration, whereas fecal samples from PA sent to the Pennsylvania Animal Diagnostic Laboratory System were cultured using a centrifugation procedure. In contrast, the estimated performance of the bacterial culture of feces using the sedimentation procedure at the University of Wisconsin Johne's Testing Center (samples from CO) seemed to be similar to the performance of the PA test using the centrifugation procedure for MAP concentration. These results confirm that the centrifugation procedure for MAP concentration can result in greater sensitivity than the sedimentation procedure in some laboratories (Eamens et al., 2000; Whitlock et al., 2000). Differences across states may also be related to the predominant strain of MAP in the herd by state, and different MAP strains may lead to diverse growth patterns in solid culture medium such as HEYM (Cernicchiaro et al., 2008).

The indexes that represented the sum of the diagnostic test conditional probabilities of correctly identifying a heavy, light, or non-shedder showed that bacterial culture performance was influenced by one of the herds. Inclusion of this herd in the analysis increased this index approximately 15% for bacterial culture and decreased approximately 6% for serum ELISA (Figure 2). This herd had the highest true prevalence of fecal shedders and a high apparent prevalence of serum ELISA-positive cows compared with the apparent prevalence of bacterial culture-positive cows (Table 1, herd 1). Although this herd may be considered as an influential outlier, it was included because this level of true prevalence is typical in some heavily infected herds.

Identification of MAP-infected cattle can be challenging. Certainly diagnostic tests are imperfect. In addition, MAP-infected cattle may remain in the herd for a long time before being identified as fecal shedders. Establishing the fecal shedding status of cattle on a regular basis and implementing management practices to reduce the transmission of MAP to susceptible cattle may be more important than identifying the actual in-

fection status of the cattle, because fecal shedders are the most important source of MAP in the environment.

For veterinary practice and for decision-making on the farm, knowing the probability of the diagnostic test result given the true fecal shedding status of the cattle is not really of interest. Predictive values are the parameters of interest in a practical context. The predictive values of bacterial culture and serum ELISA for heavy fecal shedders showed a high uncertainty. This could be explained by lower prevalence of heavy fecal shedders in these herds and the relatively small number of high bacterial culture of feces and serum ELISA test results.

These results also indicate that detecting light-shedding cows is challenging in herds with a low prevalence of shedders. In contrast, in herds with a high prevalence of fecal shedders, culling or segregating all shedding cattle can be impractical and negatively affects the economical sustainability of the herd. Another challenge is the relatively high false-negative rate of serum ELISA for detecting heavy fecal shedding cattle, as identified in this study. This is a critical problem in those herds that implemented a control program of MAP infection solely based on this diagnostic test. Using strategies that combine both tests on a regular basis may help to identify shedding cows soon after they begin shedding as part of a long-term MAP infection control program.

CONCLUSIONS

In this study, bacterial culture of feces and serum ELISA were shown as important diagnostic tools to identify heavy fecal shedders and non-shedders. In contrast, the performance of these diagnostic tests in the population of light fecal shedders was poor, and most light shedders were categorized as negative by both tests. The estimated posterior conditional distributions from this study improves interpretation of test results from bacterial culture of feces and serum ELISA, and also provides an estimation of test performance that can be used to improve models of transmission of MAP on dairy farms.

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APPENDIX

Bayesian Latent-Class Model with Conditional Independency

OpenBugs (Thomas et al., 2006) code model:

```

model
{for(i in 1:k) {
  x[i,1:9] ~ dmulti(p[i,1:9], n[i])
  p[i,1] <-P[i,1]*A[1]*C[1] + P[i,2]*B[1]*D[1] + P[i,3]*(E[1])*(F[1])
  p[i,2] <-P[i,1]*A[1]*C[2] + P[i,2]*B[1]*D[2] + P[i,3]*(E[1])*(F[2])
  p[i,3] <-P[i,1]*A[1]*C[3] + P[i,2]*B[1]*D[3] + P[i,3]*(E[1])*(F[3])
  p[i,4] <-P[i,1]*A[2]*C[1] + P[i,2]*B[2]*D[1] + P[i,3]*(E[2])*(F[1])
  p[i,5] <-P[i,1]*A[2]*C[2] + P[i,2]*B[2]*D[2] + P[i,3]*(E[2])*(F[2])
  p[i,6] <-P[i,1]*A[2]*C[3] + P[i,2]*B[2]*D[3] + P[i,3]*(E[2])*(F[3])
  p[i,7] <-P[i,1]*A[3]*C[1] + P[i,2]*B[3]*D[1] + P[i,3]*(E[3])*(F[1])
  p[i,8] <-P[i,1]*A[3]*C[2] + P[i,2]*B[3]*D[2] + P[i,3]*(E[3])*(F[2])
  p[i,9] <-P[i,1]*A[3]*C[3] + P[i,2]*B[3]*D[3] + P[i,3]*(E[3])*(F[3])

#PARAMETERS
#A1=p(Culture High | Heavy), A2=p(Culture Low | Heavy), A3=1-A1-A2=p(Culture Neg | Heavy)
#B1=p(Culture High | Light), B2=p(Culture Low | Light), B3=1-B1-B2=p(Culture Neg | Light)
#C1=p(ELISA High | Heavy), C2=p(ELISA Low | Heavy), C3=1-C1-C2=p(ELISA Neg | Heavy)
#D1=p(ELISA High | Light), D2=p(ELISA Low | Light), D3=1-D1-D2=p(ELISA Neg | Light)
#E1=p(Culture High | non-shedder), E2=p(Culture Low | non-shedder), E3=(1-E1-E2)=p(Culture
Neg | non-shedder)
#F1=p(ELISA High | non-shedder), F2=p(ELISA Low | non-shedder), F3=(1-F3- F4)=p(ELISA Neg |
non-shedder)
#P[i,1]=Prevalence of heavy at the ith farm
#P[i,2]=Prevalence of light at the ith farm
#P[i,3] = Prevalence of non-shedder at the ith farm
}

#PRIORS
P[1, 1:3] ~ ddirch(p1[ ])      #p1=c(1,1,1)
P[2, 1:3] ~ ddirch(p2[ ])      #p2=c(1,1,1)
P[3, 1:3] ~ ddirch(p3[ ])      #p3=c(1,1,1)
P[4, 1:3] ~ ddirch(p4[ ])      #p4=c(1,1,1)
P[5, 1:3] ~ ddirch(p5[ ])      #p5=c(1,1,1)
P[6, 1:3] ~ ddirch(p6[ ])      #p6=c(1,1,1)
P[7, 1:3] ~ ddirch(p7[ ])      #p7=c(1,1,1)
P[8, 1:3] ~ ddirch(p8[ ])      #p8=c(1,1,1)
P[9, 1:3] ~ ddirch(p9[ ])      #p9=c(1,1,1)
P[10, 1:3] ~ ddirch(p10[ ])     #p10=c(1,1,1)
P[11, 1:3] ~ ddirch(p11[ ])     #p11=c(1,1,1)
P[12, 1:3] ~ ddirch(p12[ ])     #p12=c(1,1,1)
P[13, 1:3] ~ ddirch(p13[ ])     #p13=c(1,1,1)
P[14, 1:3] ~ ddirch(p14[ ])     #p14=c(1,1,1)
P[15, 1:3] ~ ddirch(p15[ ])     #p15=c(1,1,1)
P[16, 1:3] ~ ddirch(p16[ ])     #p16=c(1,1,1)
P[17, 1:3] ~ ddirch(p17[ ])     #p17=c(1,1,1)
A[1:3] ~ ddirch(m1[ ])          #m1=c(95,4,1),
B[1:3] ~ ddirch(m2[ ])          #m2=c(5,55,40)
C[1:3] ~ ddirch(m3[ ])          #m3=c(60,30,10)
D[1:3] ~ ddirch(m4[ ])          #m4=c(1,24,75)
E[1:3] ~ ddirch(m5[ ])          #m5=c(1,1,98)
F[1:3] ~ ddirch(m6[ ])          #m6=c(1,1,98)
}

```