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Effects of prevalence and testing by enzyme-linked immunosorbent assay and fecal culture on the risk of introduction of *Mycobacterium avium* subsp. *paratuberculosis*–infected cows into dairy herds

Tim E. Carpenter,[1] Ian A. Gardner, Michael T. Collins, Robert H. Whitlock

Abstract. A stochastic simulation model was developed to assess the risk of introduction of *Mycobacterium avium* subsp. *paratuberculosis* infection into a dairy herd through purchase of female replacement cattle. The effects of infection prevalence in the source herd(s), number of females purchased, and testing by enzyme-linked immunosorbent assay (ELISA) alone or ELISA and fecal culture as risk mitigation strategies were evaluated. Decisions about negative test results were made on a lot and individual basis. A hypothetical dairy herd, free from *M. a. paratuberculosis,* which replaced 1 lot (10, 30, or 100) of cows per year, was considered. Probability distributions were specified for the sensitivities and specificities of ELISA and fecal culture, the proportion of infected herds and within-herd prevalence for randomly selected replacement source herds (high prevalence) and herds in level 2 (medium prevalence) and level 3 (low prevalence) of the Voluntary Johne's Disease Herd Status Program (VJDHSP). Simulation results predicted that 1–56% of the lots had at least 1 *M. a. paratuberculosis*–infected cow. Assuming that ELISA sensitivity was 25%, simulation results showed on a lot basis that between 0.4% and 18% and between 0.1% and 9% were predicted to have at least 1 infected cow not detected by ELISA and by a combination of ELISA and fecal culture, respectively. On an individual cow basis, between 0.1% and 8.3% of ELISA-negative cattle in ELISA-positive lots were estimated to be infected. In both the lot and individual analyses, the probability of nondetection increased with larger lot sizes and greater prevalence. Sensitivity analysis indicated that the effect of a lower ELISA sensitivity (10%) was a variable decrease in mean detection probabilities for all combinations of prevalence and lot size. The benefit of testing introduced cattle with ELISA alone or in combination with fecal culture was found to be minimal if cows were purchased from known, low-prevalence (level 3) herds. The value of testing by ELISA alone or in combination with fecal culture was greatest in high-prevalence herds for all lot sizes. Testing of random-source cattle, bought as herd replacements, can partially mitigate the risk of introduction of *M. a. paratuberculosis* but not as well as by using low-prevalence source herds (level-3 VJDHSP), with or without testing.

Mycobacterium avium subsp. *paratuberculosis* infection (commonly termed paratuberculosis or Johne's disease) occurs in dairy and beef herds throughout the United States and the rest of the world. Based on results of the National Animal Health Monitoring System (NAHMS) Dairy '96 study, 22% of US dairy herds were estimated to have *M. a. paratuberculosis*–infected cattle.[25] However, this is a conservative herd prevalence estimate, especially among large herds, which have a higher risk of infection.[26] In the NAHMS study, underestimation of herd prevalence (false-negative herds) probably occurred because testing of 30 females failed to detect infection in low-prevalence herds. In contrast to the US estimate, 50% to 70% of Danish[15] and Dutch dairy herds,[9,14] respectively, are reported to be *M. a. paratuberculosis* infected.

Trade in infected cattle is believed to be the most important source of among-herd transmission of *M. a. paratuberculosis;* hence, a strategy that herd owners can use to minimize the risk of introducing infection is to only purchase replacement females from herds that are free (or have a low prevalence) of infection. In response to growing concern in the US cattle industries about the economic importance of paratuberculosis, the Voluntary Johne's Disease Herd Status Program (VJDHSP) was developed in 1998. The program provides guidelines for the operation of a scientifically sound program to identify herds with a low probability of infection and a low within-herd prevalence of *M. a. paratuberculosis.* The model program was developed to provide greater similarity and equity among different state herd certification programs for *M. a. paratuberculosis.*[1,2] In brief, the VJDHSP has 4 levels for test-negative herds, each with different testing and biosecurity requirements. Progression from

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level 1 to level 4 in the VJDHSP is associated with a higher probability that a herd is free of infection and lower within-herd prevalence if the herd were infected. Combinations of enzyme-linked immunosorbent assay (ELISA) and fecal culture are used in the program, and a recent study indicated that the sensitivity of herd classification in the VJDHSP (sample size of 30) ranged from 33% to 84%; whereas the herd-level sensitivity of ELISA, if it were used without confirmation by fecal culture, ranged from 70% to 93%. In addition, without fecal culture, the herd-level specificity was predicted to decrease to 11%.[27]

The sensitivity of the ELISA for the detection of serum antibodies to *M. a. paratuberculosis* in individual cows has been evaluated extensively.[3–6,13,16,19,21,22,28] Although there is conjecture about estimated values and the appropriate reference test, current evidence indicates that ELISA sensitivity is highly dependent on the stage of *M. a. paratuberculosis* infection (stage I: preclinical, not shedding bacteria in feces; stage II: preclinical, shedding bacteria in feces; or stage III: clinical, shedding bacteria in feces). Estimates range from 15% (stage I) to 85% (stage III).[6] In stage II, ELISA sensitivity is positively correlated with the number of *M. a. paratuberculosis* shed in feces.[22,28] In addition, ELISA sensitivity can decrease over time because of a change in the spectrum of disease caused by the culling of ''heavy'' fecal shedders.[28] Finally, the sensitivity of the ELISA is dependent also on the designated cutoff value(s) for interpretation of test results, and there are several variants of the ELISA in different laboratories.

The sensitivity of a single fecal culture in stage-II infection is considered by many authors to be greater than that of ELISA; however, the sensitivity is dependent on both the numbers of *M. a. paratuberculosis* shed in feces[22,28] and the culture method used.[7,20] Estimates of the sensitivity of a single fecal culture by conventional methods range from 30% to 50%.[12,28]

The objective of the present risk assessment was to develop a simple model for estimation of the risk of introduction of *M. a. paratuberculosis* infection into a dairy herd associated with purchase of replacement females. The effects of among- and within-herd prevalence of *M. a. paratuberculosis* infection in source herds, number of adult females purchased, and testing of purchased cattle as a risk mitigation strategy were evaluated. Two possible testing scenarios were considered: use of ELISA only and use of a combination of ELISA and fecal culture.

Methods

Model herd and diagnostic testing. A hypothetical dairy herd, free from *M. a. paratuberculosis,* that replaced 10, 30, or 100 of its cows per year was considered. The manager has the option of buying cows or heifers from multiple source herds of known or unknown *M. a. paratuberculosis* status. For the purpose of this study, it was assumed that a lot of cattle either came from a single herd or multiple herds of an identical *M. a. paratuberculosis* status. The manager can purchase the cows untested or can use ELISA individually or in combination with fecal culture on all purchased cattle. For purchases from source herds of unknown status or level-2 or level-3 herds in the VJDHSP program, 3 possible purchase options of cows 2 yr of age or older were considered: 1) no testing, 2) testing and introduction of ELISA-negative cattle, and 3) testing and introduction of cattle that were negative on both ELISA and fecal culture. For the last option, it was assumed that testing by ELISA was done first because of its rapidity and lower cost. Only cattle that tested negative on the ELISA were then tested by fecal culture. For tested cattle, decisions about introduction were assumed to be made either on an individual animal basis (reject test-positive cattle, and according to this assumption, the risk of introduction of infected animals was estimated if test-negative cattle were purchased) or on a lot basis (reject the lot if 1 or more animals tested positive).

For the purpose of this analysis, it was assumed that there was 1 purchase/yr; however, the results may pertain to multiple purchases during a longer time period as well. For simplicity, all introduced infected females were assumed to be subclinically infected and shedding *M. a. paratuberculosis* in feces, i.e., stage-II infection. Monte Carlo simulations were used to generate probability distributions for the between- and within-herd and lot prevalence of *M. a. paratuberculosis.* Monte Carlo simulations may be thought of as a random sampling technique that chooses an event based on the weight or probability of that event occurring. For example, if 22% of the herds were infected, on average 22% of the simulations will presume that females are being purchased from an infected herd. The method continues to be used to determine the infection and test status of each individual in a lot. All outcomes were determined by the probability distribution of the events, as described below.

Probability distributions for model inputs. Because estimates of ELISA sensitivity in the published literature vary greatly, MTC and RHW, who separately have evaluated the ELISA,[3,4,22,28] gave their expert opinion about test accuracy. The experts synthesized evidence from their own and others works into both the most likely value (which was equated to the mode) and the value that they were 95% sure that sensitivity of the commercial ELISA[a] was below when used in typical adult dairy cows in stage-II infection. Cows in stage-II infection shed few, moderate, or many *M. a.*

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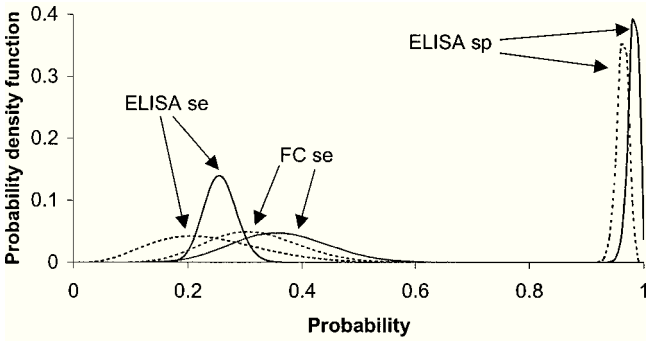

Figure 1. Probability distributions for ELISA sensitivity (se) and specificity (sp) and fecal culture (FC) sensitivity for the *Mycobacterium avium paratuberculosis* risk introduction model based on opinions of 2 subject matter experts (expert 1, solid lines; expert 2, dashed lines). (Specificity of FC was not fixed exactly at 100% [not shown] because of the possibility that *M. a. paratuberculosis* might be transiently present in feces without concurrent infection.[23] However, the likelihood of this phenomenon was considered to be low, and the expert estimations of FC specificity were >0.995 with a probability of at least 99%.)

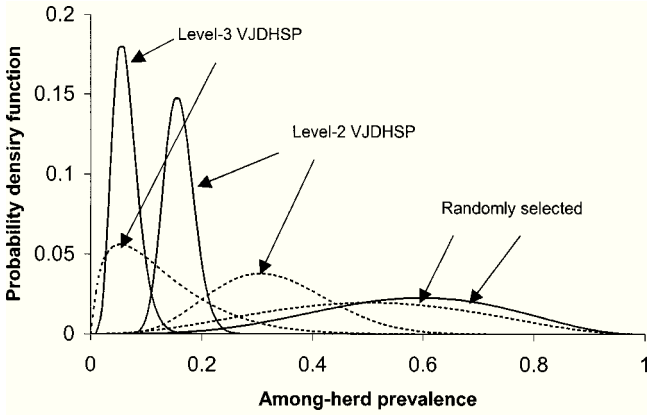

Figure 2. Among-herd prevalence distributions for 3 herd types (level-3 VJDHSP, level-2 VJDHSP, and randomly selected [national] herds) for the *Mycobacterium avium paratuberculosis* risk introduction model based on opinions of 2 subject matter experts (expert 1, solid lines; expert 2, dashed lines).

paratuberculosis in feces; therefore, their estimate was based on a consideration of the proportion of cows in each of these ''shedding'' categories in a typical herd and the sensitivity for each category. Similar inputs were obtained for the specificity of ELISA, the sensitivity and specificity of fecal culture, the proportion of infected herds and within-herd prevalence for a randomly selected national herd and for herds in levels 2 and 3 of the VJDHSP. For parameters that had a mode of >0.5, the experts indicated the value that they were 95% sure that the parameter exceeded because accurate characterization of the shape of the distribution is more readily achieved in that situation when the lower rather than the upper limit is used. For the 2 values that the expert specified for each input, a beta distribution was fitted[b] that most closely matched the expert's opinion (Figs. 1–3). The beta distribution was selected for all modeled distributions and is appropriate for modeling binomial probabilities such as sensitivity, specificity, and prevalence because it is unimodal and flexible to use. The parameters (α and β) of the beta distribution determine its shape and reflect the certainty that the expert has about the modal value for the distribution. Beta distributions can be made more concentrated (or more diffuse) about a single mode by increasing (decreasing) the values of α and β by the same magnitude. Distributions were graphed as a final check on the correctness of the provided estimates.

Scenarios and simulations. Three primary scenarios were evaluated: purchases from a group of low- (level 3), medium- (level 2), or high-prevalence (randomly selected) herds. For each of these 3 scenarios, there were 3 options (no testing, testing by ELISA, and testing by ELISA and fecal culture) and 3 lot sizes (10, 30, and 100). Initial simulations were performed using inputs from the first expert (MTC, Figs. 1–3). A total of 10,000 iterations for each of the 27 alternative scenarios were performed. Simulations were done in @RISK,[c] a simulation add-in for Excel.[d]

A sensitivity analysis was done to evaluate the effects of 3 factors: use of inputs from the second expert (RHW) compared with the first expert (MTC); a lower ELISA sensitivity (10%), which might apply when the replacement females were heifers rather than cows; and a dependence between the sensitivity of the ELISA and fecal culture, which might occur because the combined sensitivity of the 2 tests used in series is less than that predicted based on an assumption of independence.[8] The conditional sensitivity of fecal culture in the subpopulation of ELISA-negative cattle was set as 0.3, compared with its overall value of 0.35 in untested cattle in stage-II infection. The value was

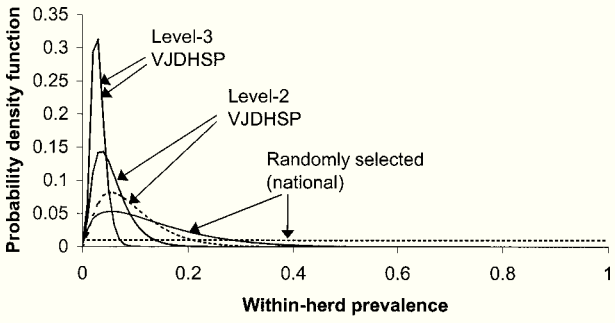

Figure 3. Within-herd prevalence distributions for 3 herd types (level-3 VJDHSP, level-2 VJDHSP, and randomly selected [national] herds) for the *Mycobacterium avium paratuberculosis* risk introduction model based on opinions of 2 subject matter experts (expert 1, solid lines; expert 2, dashed lines). (Note that distributions for level-3 herds were virtually identical.)

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Table 1. Percentage of *Mycobacterium avium paratuberculosis*–infected lots (at least 1 infected female) and numbers of infected cows per infected lot at 3 prevalence scenarios and 3 lot sizes, with priors of expert 1.

Testing option	Level 3 herds*			Level 2 herds†			National herds‡		
	10	30	100	10	30	100	10	30	100
None									
Infected lots (%)	1.5	3.3	5.5	5.4	10.5	14.7	36.2	49.9	56.0
Median number infected per lot	1	1	3	1	2	4	2	3	10
Maximum number infected per lot	2	4	9	4	8	21	8	19	58
ELISA§									
Undetected infected lots (%)	0.9	1.2	0.4	3.3	3.4	0.7	18.1	10.5	1.1
Median number infected per lot	1	1	2	1	2	3	1	2	4
Maximum number infected per lot	2	3	6	3	7	10	7	14	14
ELISA and FC‖									
Undetected infected lots (%)	0.5	0.4	0.1	1.6	1.0	0.1	8.7	2.4	0.1
Median number infected per lot	1	1	2	1	1	3	1	2	2
Maximum number infected per lot	2	2	3	3	4	6	6	13	9

* Within-herd prevalence for level-3 herds: mode = 2%.
† Within-herd prevalence for level-2 herds: mode = 3%.
‡ Within-herd prevalence for national herds: mode = 5%.
§ ELISA sensitivity: mode = 25%.
‖ Fecal culture (FC) sensitivity: mode = 35%.

chosen based on analysis of Johne's disease repository data[18] and on experience of one of the authors (IAG) in the assessment of test dependence.

Posttest probabilities. Posttest probabilities (predictive values) of infection were calculated at the individual and lot levels to better interpret negative test results for the 2 testing scenarios. For individual interpretation, posttest probabilities depend on sensitivity, specificity, and pretest probability (prevalence) and lot-level probabilities depend on these 3 factors, the lot size, and the proportion of infected lots.[11] At a lot level, the probability of at least 1 *M. a. paratuberculosis*–infected female being present, given that all females in the lot were test negative, is 1 minus negative predictive value (1 − NPV), which has an interpretation similar to that for individual test results. For example, if 1 − NPV was 0.10 for lot-level results, this finding indicates that 1 in 10 test-negative lots of the designated size has at least 1 infected female.

Results

Simulation results predicted that 1–56% of untested lots had at least 1 *M. a. paratuberculosis*–infected cow (Table 1). Lots from randomly selected (national) herds had a higher proportion of infected lots (for all lot sizes) than lots from level-2 herds, which were higher than lots from level-3 herds. Within each herd prevalence category, larger lot sizes increased the risk of having infected animals. The median number of infected cows in infected lots ranged from 1 to 4, except in lots of 100 cows from randomly selected (national) herds, where the median was 10 infected cows. The maximum number of infected cows in infected lots ranged from 2 to 58.

Lot-level posttest probabilities. Assuming an ELISA sensitivity of 25% (mode for expert 1), the model predicted that 36–98% of infected lots would be detected because they had at least 1 ELISA-positive cow (Table 2). Probabilities of detection increased with larger lot sizes and were higher for randomly selected (national) herds than for level-2 herds, which were higher than that for level-3 herds. The combination of ELISA and fecal culture detected more (69–99.9%) infected lots for all combinations of lot size and within-herd prevalence. Probabilities of detection increased with larger lot sizes and were higher for randomly selected herds than for level-2 herds, which were higher than that for level-3 herds. The net effect of these predicted detection probabilities was reflected in the percentage of undetected, infected lots for both testing options (Table 1). Depending on the source herd, 1–18% of all lots were predicted to have infected cows not detected by the ELISA and 0.1–9% not detected by a subsequent fecal culture (Table 1). For both testing strategies, the proportion of lots with more infected animals than test-positive animals increased with increasing prevalence and also with larger lot sizes within each prevalence category (data not shown). Some positive lots were correctly detected as infected because of false-positive tests on noninfected animals (mode for ELISA specificity for expert 1 was 0.98) in the lot even when truly infected animals tested negative.

Cow-level posttest probabilities. If the decision

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Table 2. Percentage of *Mycobacterium avium paratuberculosis*–infected lots detected for 3 prevalence scenarios and 3 lot sizes, with priors of expert 1.

	Level-3 herds*			Level-2 herds†			National herds‡		
Testing option	10	30	100	10	30	100	10	30	100
ELISA sensitivity = 25%									
ELISA	35.6	64.7	92.6	40.0	68.0	95.0	49.9	79.0	98.0
ELISA and FC§	68.5	88.7	99.1	70.1	90.1	99.4	77.6	95.1	99.9
ELISA sensitivity = 10%									
ELISA	26.7	56.1	91.1	27.7	57.1	91.9	32.4	64.0	94.1
ELISA and FC§	63.0	86.5	98.9	65.3	88.1	99.3	71.8	93.2	99.8

* Within-herd prevalence for level-3 herds: mode = 2%.
† Within-herd prevalence for level-2 herds: mode = 3%.
‡ Within-herd prevalence for national herds: mode = 5%.
§ Fecal culture (FC) was applied to all cattle in ELISA-negative lots.

were made on an individual cow basis, instead of lot basis, the dairyman may consider retaining test-negative cows present in a test-positive lot once the reactors were removed. Because both ELISA and the combination of ELISA and fecal culture were imperfectly sensitive, variable numbers of test-negative cows were predicted to be truly infected. The probability that a test-negative cow was actually infected (1 − NPV) after test-positive cows were removed from the lot increased with both increasing lot size and higher herd prevalence and ranged from 0.1% to 8% (Table 3). The highest value for 1 − NPV occurred in randomly selected (national) herds, where 6–8% of ELISA-positive lots were predicted to have ELISA-negative infected cows (Table 3). The use of fecal culture in addition to ELISA reduced the simulated value of 1 − NPV by an average of approximately 50% for all combinations of lot size and herd prevalence category and as much as 90% for ELISA-negative and fecal culture–positive lots.

The simulated percentage of lots testing ELISA positive ranged from 21% to 93%, increasing with lot size and within-herd prevalence (Table 4). If the lot was ELISA negative and tested with fecal culture, the prevalence of fecal culture–positive lots ranged from 6% to 24%, being lowest for the largest lot size as a result of the relatively small number of ELISA-negative 100-cow lots. The percentage of ELISA-negative and fecal culture–negative lots ranged from 1% to 63%. The smallest percentages occurred in the 100-cow lots and largest percentages in the 10-cow lots. The number of infected cows not detected by ELISA varied across within-herd prevalence levels and lot sizes. Although the number of undetected, infected cows was small in lots from level-2 and level-3 herds, 40% of the 100-cow lots from randomly selected (national) herds were expected to have at least 5 infected cows not detected by ELISA (Table 5), compared with less than 1% and approximately 4% in the 100-cow lots from level-3 and level-2 herds, respectively.

Sensitivity analysis. The effect of change in the source of expert opinion was minimal, and the same relationships were mostly evident. Because the second expert (RHW) allowed for larger within-herd prevalence in national herds, the mean number of simulated infected cattle per infected lot was about 4-fold higher. In addition, he estimated a lower modal value for specificity (0.96). This resulted in a greater detection probability for larger lots from national herds (87% and 95% for lot sizes of 30 and 100, respectively) when

Table 3. Posttest probability (%) of *Mycobacterium avium paratuberculosis* infection (1 − NPV) for ELISA-negative females in test-positive (ELISA+ cattle removed) and test-negative lots, at 3 prevalence levels and 3 lot sizes with priors of expert 1.

Testing option and level		Level-3 herds*			Level-2 herds†			National herds‡		
Lot	Individual	10	30	100	10	30	100	10	30	100
ELISA+	ELISA−	0.14	0.14	0.13	0.72	0.66	0.60	8.29	7.33	6.03
ELISA−	ELISA−	0.12	0.10	0.07	0.50	0.42	0.21	4.38	2.68	0.68
ELISA−, FC+§	ELISA−, FC−	0.08	0.07	0.05	0.49	0.39	0.15	4.52	2.57	0.55
ELISA−, FC−	ELISA−, FC−	0.08	0.06	0.03	0.31	0.20	0.10	2.37	1.00	0.17

* Within-herd prevalence for level-3 herds: mode = 2%.
† Within-herd prevalence for level-2 herds: mode = 3%.
‡ Within-herd prevalence for national herds: mode = 5%.
§ Fecal culture (FC) was applied to all ELISA-negative cattle.

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Table 4. Distribution (%) of ELISA and fecal culture (FC) test results to identify *Mycobacterium avium paratuberculosis* infection at 3 prevalence levels and 3 lot sizes with priors of expert 1.

	Level-3 herds*			Level-2 herds†			National herds‡		
Testing result	10	30	100	10	30	100	10	30	100
ELISA+	20.9	50.0	86.6	21.8	51.5	87.5	31.1	63.9	92.7
ELISA−	79.1	50.0	13.4	78.2	48.5	12.5	68.9	36.1	7.3
ELISA−, FC+§	16.2	23.5	10.6	16.6	23.2	9.9	20.2	19.8	5.9
ELISA−, FC−	62.9	26.5	2.8	61.6	25.3	2.6	48.7	16.3	1.4

* Within-herd prevalence for level-3 herds: mode = 2%.
† Within-herd prevalence for level-2 herds: mode = 3%.
‡ Within-herd prevalence for national herds: mode = 5%.
§ Fecal culture was applied to all ELISA-negative cattle.

lot-level interpretation of ELISA results was used. The effect of a reduction in ELISA sensitivity from 25–10% was a decrease in mean detection probabilities of 16% for all combinations of prevalence and lot size (Table 2). The decrease ranged from 2–4% to 25–35% for 100-cow and 10-cow lots, respectively. The effect of the lower ELISA sensitivity when combined with fecal culture was a mean reduction of 3%. The effect of dependence on the sensitivities of ELISA and fecal culture was typically less than a 2% reduction in percentage of infected lots detected (data not shown) compared with those values obtained when conditional independence of the tests was assumed (Table 2).

Discussion

Previous studies have shown that prevalence in the source herd, sensitivity of the tests used, and number of animals purchased affect the risk of introduction of infection when there are false-negative test results.[10,24] However, these studies did not consider the effects of uncertainty in estimates of test sensitivity and prevalence or the use of 2 tests that might be dependent when used in combination as was done in this study.

The benefit of testing introduced cattle with ELISA alone or in combination with fecal culture was found to be minimal if cows were purchased from known, low-prevalence (level 3) herds. In addition, even if the lot were infected, the number of purchased infected animals was lower when selections were made from these herds compared with level-2 and national herds. The value of testing by ELISA alone or in combination with fecal culture increased greatly in randomly selected high-prevalence herds. When cows were purchased from randomly selected herds, lot-level interpretation of ELISA and a combination of ELISA and fecal culture results provided a greater reduction in risk than individual-level interpretation. An alternative approach to this problem that decreases risk of introduction of infection even further, but beyond the scope of this article, would be to assume that individual lots were purchased from a single herd, and the entire lot would be either accepted or rejected based on test results.

Table 5. Cumulative percentage of lots in which X *Mycobacterium avium paratuberculosis*–infected animals were not detected, using ELISA as a screening method with the sensitivity prior for expert 1, for 3 levels of prevalence and 3 lot sizes (note that total percentages exceed those reported in Table 1 for the percentage of lots undetected by ELISA because all infected animals were not detected in some of the infected lots).

No. infected animals not	Level-3 herds*			Level-2 herds†			National herds‡		
detected X	10	30	100	10	30	100	10	30	100
1	1.1	1.8	1.7	3.6	4.8	2.6	17.4	11.7	3.4
2		2.5	3.2	4.4	7.3	5.5	25.7	21.4	7.3
3		2.6	4.0		8.4	7.7	29.3	29.2	11.2
4			4.6		8.8	9.7	30.5	35.0	15.3
5			4.8		8.9	11.1	30.9	39.0	19.3
6			5.0			12.1	31.0	42.1	23.1
7						12.8		44.1	26.4
8						13.2		45.3	29.6
9						13.5		46.0	32.5
≥10						14.0		47.1	55.2

* Within-herd prevalence for level-3 herds: mode = 2%.
† Within-herd prevalence for level-2 herds: mode = 3%.
‡ Within-herd prevalence for national herds: mode = 5%.

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Although findings reported in this study are specific for lots of 10, 30, or 100, they emphasize the importance of having a prevalence estimate for the herd of origin before assessing the risk of disease introduction and the need for testing. If culling rate is independent of herd size and all replacement females are introduced from outside sources, then larger herds, if they were free of *M. a. paratuberculosis,* are clearly at greater risk of introducing infected animals. For example, at a typical industry culling rate of 30%, a dairyman with a herd of 1,000 cows would introduce 300 replacement females per annum compared with 30 females for a herd of 100 cows. Hence, large herds would need to adopt strategies such as purchase from low-prevalence herds to mitigate against this increased risk. Similarly, if a new herd of 1,000 cows were established from the same multiple sources, there would be a higher probability that the new herd would be infected at start-up than a similar herd of 100 cows purchased from the same sources.

Use of probability estimates (1 − NPV) and *M. a. paratuberculosis* production loss information allows a dairyman to make an economically rational decision regarding selection of source animals and the need for testing them before entry into his herd. Although results presented in this study provided insight into the effects of purchasing practices and testing, the financial implications of such actions need to be evaluated in a decision analysis framework.[17] However, if cows of the same quality can be purchased from level-3 herds at a cost comparable with purchases from herds in level 2 of the VJDHSP or untested herds, then the findings of the authors indicate that such a choice is well justified.

Based on the simulation results, the probability of a producer purchasing an infected lot during a given time period may be evaluated. For illustrative purposes, it was assumed that a producer was embarking on a Johne's disease control program and interested in the probability of not introducing an *M. a. paratuberculosis*–infected cow into his herd. Two alternative testing practices (no test and ELISA lot test), 2 lot size purchases (10 cows and 100 cows), and 2 source herds (level 3 and random [national]) were considered. If the buyer were considering purchasing replacement cattle from level 3 (within-herd prevalence mode = 2%) without testing the lot, it was estimated that he would have a 7% to 25% chance of introducing the infection into his herd within 5 years if his annual lot size were 10 or 100 cattle, respectively. On the other hand, if he purchased from random (national) herds (within-herd prevalence mode = 5%), it was estimated that he would have an 89% to 98% chance of introducing the infection into his herd within 5 years, if his annual lot size were 10 or 100 cattle, respectively. If lots were tested using ELISA, the probabilities of introducing infected cows in 5 years were reduced by approximately one third to one-half of the nontest values for lots of 10–30 cows. Probabilities were decreased by more than 90% for 100-cow lot purchases made from level-3 and national herds (2% and 5%, respectively). These findings quantify the importance of purchasing from known low-prevalence herds and the use of ELISA and fecal culture by herd owners interested in establishing or maintaining a herd free of *M. a. paratuberculosis.*

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Sources and manufacturers

a. IDEXX Laboratories, Westbrook, ME.
b. BayesFreecalc. Available at: www.epi.ucdavis.edu/diagnostictests/.
c. Palisade Corporation, Newfield, NY.
d. Excel, Microsoft Corporation, Redmond, WA.

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