Assessment of test results when using a commercial enzyme-linked immunosorbent assay for diagnosis of paratuberculosis in repeated samples collected from adult dairy cattle

Heather L. Hirst, DVM, MS; Franklyn B. Garry, DVM, MS, DACVIM; M. D. Salman, BVMS, PhD, DACVPM

Objective—To determine the proportion of adult cattle that change test status when an ELISA for antibodies against *Mycobacterium avium* subsp *paratuberculosis* (MAP) is used to assay samples collected twice at variable intervals and to determine whether cows with an initial strong positive result were more likely to maintain positive status, compared with all cows with an initial positive result.

Design—Cross-sectional observational study.

Animals—3,757 adult dairy cattle.

Procedure—Serum samples were obtained twice from cattle at intervals ranging from 77 to 600 days between collections. Samples were tested with an ELISA for detection of antibodies to MAP.

Results—Of 157 cattle with initial positive results (value for the sample divided by the value for positive-control serum [S/P] ≥ 0.25), 62 (39.5%) had negative results for the second sample. Of 71 cattle with an initial S/P value ≥ 0.40, 13 (18.3%) had a negative result (S/P < 0.25) for the second sample. Of 33 cattle with an initial S/P ≥ 0.70, 3 (9.1%) had a negative result (S/P value < 0.25) for the second sample. Interval between collection of samples did not affect results.

Conclusions and Clinical Relevance—Many cows changed ELISA status between samples collected at variable intervals. Cows with an initial high S/P value (≥ 0.70) were more likely to maintain positive status than cows classified as positive on the basis of cutoff values of ≥ 0.25 or ≥ 0.40. Veterinarians should expect variability in ELISA results when repeated testing of cattle is used as part of an MAP control program. (*J Am Vet Med Assoc* 2002;220:1685–1689)

Infection with ***Mycobacterium avium* subspecies *paratuberculosis*** **(MAP)**, which is commonly known as Johne's disease, causes chronic enteritis in dairy cattle. Dairy herds that contain cows infected with MAP may have substantial economic losses attributable to decreased milk production in subclinically affected cattle.[1-5] Other losses are incurred as a result of decreased value of culled cows and increased replacement costs on dairies with infected cattle.[6] Testing and subsequent culling of cattle with positive test results in conjunction with management modifications have been proposed as methods to reduce the overall amount of MAP infection on dairies.[7]

Diagnosis of infected cattle is challenging because of the limited sensitivity and imperfect specificity of available tests. Sensitivity for a commercially available ELISA[a] ranges from 15 to 45% in subclinically affected cattle, and specificity for that ELISA ranges from 96.8 to 99%.[8,9] Costs to test each sample are between $4 and $5, and it requires 1 to 3 days to obtain results with this ELISA. Sensitivity and specificity for microbial culture of fecal samples in cows without clinical signs of disease are 25 to 50% and 100%, respectively.[10,11] Cost for culturing of fecal samples is $15 to $20/cow, and it requires up to 4 months to obtain results.

The ELISA[a] used in the study reported here has been validated as a useful test for measuring antibody response to MAP infection in cattle and has been recommended by the United States Animal Health Association for identification of herds at low risk for MAP infection.[12] Many dairies do not aggressively cull ELISA-positive cattle, because this ELISA was not designed for use in testing individual cattle. Such dairies may obtain additional samples from cows with positive results as part of a routine program to monitor prevalence of ELISA-positive cows within the herd. When results from an individual cow are discrepant for repeat samples, producers and veterinarians become frustrated and may be discouraged from continued use of the ELISA in control programs.

Investigators as well as producers and veterinarians have encountered problems with cows whose results fluctuate between positive and negative status when serial serum samples are tested by use of the ELISA.[13] Because results for the ELISA are expressed as a **ratio for the value of the sample to the value of a positive-control serum (S/P)**, as determined by measurement of **optical density (OD)**, implementation of multiple cutoff values may be useful for reducing the degree of fluctuation in status for a specific cow that is evident when cattle are sampled repeatedly.[5,8,14]

The manufacturer of the ELISA[a] recommends a single cutoff value (S/P ≥ 0.25) for classification of positive results; negative results would be S/P values < 0.25. Collins and Sockett[8] calculated likelihood ratios for cows that had a range of OD values and

From the Departments of Clinical Sciences (Hirst, Garry, Salman) and Environmental Health (Salman), College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523.

Supported in part by funds from the Colorado Agricultural Experiment Station and contributions from Colorado Dairy Producers.

Address correspondence to Dr. Hirst.

found that cows with intermediate OD values were more likely to be truly infected than cows with OD values in the negative range. Infection status was confirmed after a group of cows was followed to necropsy or developed clinical signs.[8,10] It has been suggested that an intermediate category be used as a suspect range for reporting results to producers.[14]

Likelihood ratios for cows with various ELISA results have not been defined in the western United States, an area in which the ecologic characteristics of MAP and, consequently, the performance of the ELISA may differ from that described in other parts of the country. A few studies of ELISA tests of samples obtained from cattle on dairies in the western United States can be found in the literature, including our report on the seroprevalence of MAP infection in dairies in Colorado.[b] The objective of the study reported here was to determine the proportion of cattle that have a change in ELISA test results when samples were collected twice at various intervals. A second objective was to determine whether cows with an initial strong positive test result were more likely to maintain positive status, compared with all cows with an initial positive result.

Pessimistic responses from the dairy industry could possibly be avoided if the degree of variability in test results could be predicted for cows that are tested more than once at varying intervals. In addition, the use of higher S/P cutoff values for a positive test result would improve our ability to predict the outcome of serial testing.

Materials and Methods

Animals—Cattle used for repeat sample collection in the study originated from 11 dairy herds in Colorado. In each herd, within-herd seroprevalence was determined during 1999 and 2000 by use of the ELISA[a] on samples obtained from all cattle ≥ 2 years old. Two-year-old heifers were excluded from sample collection if they had not yet given birth to a calf. This sample collection procedure was performed as part of a survey of dairy herds in Colorado.[b,c]

Following whole-herd ELISA testing, samples were again collected from cows on these 11 dairies at various intervals and for various reasons. A common recommendation for herds interested in control programs for MAP is to establish a protocol of repeat sample collection for all cows at the time that a veterinarian examines cattle to confirm pregnancy status. At that time, cattle are sorted and identified by use of leg bands or colored ear tags as being ELISA-positive or -suspect prior to the nonlactating period and periparturient (maternity) period. Cattle that are ELISA-positive prior to entering the nonlactating period may be closely monitored during parturition, and colostrum from these cattle can be discarded to decrease the risk of disease transmission.

Two farms routinely collected samples from cows prior to entering the nonlactating period. One farm obtained samples from cows prior to entering the nonlactating period and from groups of cows previously identified as ELISA-positive or -suspect. One farm obtained samples from groups of culled cows, from cows prior to entering the nonlactating period, from groups of cows previously identified as ELISA-positive or -suspect, and from cows on the basis of results of 2 whole-herd tests performed 1 year apart. One farm obtained serial samples from groups of culled cows and from cows on the basis of results of 2 whole-herd tests performed 1 year apart. Two farms obtained samples from groups of cows previously identified as ELISA-positive or -suspect and from cows on the basis of results of 2 whole-herd tests performed 1 year apart. On 4 farms, samples were obtained from small groups of cows previously identified as ELISA-positive or -suspect at various intervals.

Although some dairies used more than 1 reason to determine the cows from which subsequent samples would be obtained, each cow was used only once (1 initial and 1 repeat sample) in the data set collected for this study. The initial serum sample for each cow was collected at the whole-herd ELISA test during 1999 and 2000. The second sample was collected at a variable interval after the initial sample. When more than 1 repeated serum sample was collected from an animal, the first repeat sample (ie, shortest interval after initial herd test) was used for the data set in this study. Results for the 2 samples were identified as initial S/P and repeat S/P.

ELISA procedure and categories—Repeat serum samples were collected by herd managers, dairy personnel, or veterinarians at variable intervals and for the various aforementioned reasons. Sera were tested by use of the ELISA at the same laboratory and by the same technician. Testing was performed in accordance with the manufacturer's protocol,[a] except that samples were not assayed in duplicate wells in an effort to reduce cost to the dairy producers. Results of the ELISA were reported as the S/P value. Sera with S/P values of 0.00 to 0.09 were classified as negative, whereas S/P values of 0.10 to 0.24 were classified as suspect, and S/P values ≥ 0.25 were classified as positive. The manufacturer[a] did not encourage or discourage use of the suspect classification.

Data analysis—Results of repeat serum samples from all cattle were combined, regardless of their herd of origin. Subsequently, to evaluate whether the ELISA results differed among herds with various herd seroprevalence, the following 2 groups of cattle were established for describing results of repeat serum samples: cows originating from dairies with **high within-herd seroprevalence** (**HPH**; ≥ 5% of cows were seropositive) and cows originating from dairies with **low within-herd seroprevalence** (**LPH**; < 5% of cows were seropositive). It was confirmed that each herd in the HPH group contained cows with MAP infection by use of culture of fecal samples or necropsy and by histopathologic diagnosis. Some herds in the LPH group had cows with positive results for bacteriologic culture of fecal samples, but others did not have confirmation of MAP infection.

We then increased the cutoff value for determining seropositive status for the initial S/P to ≥ 0.40 and ≥ 0.70. The frequency with which cows converted to seronegative status (S/P < 0.25) on the repeat S/P was recorded.

An ANCOVA was used to compare mean number of days between repeat samples for 9 categories of cows; within-herd prevalence (LPH or HPH) was used as the covariate. The 9 categories were defined on the basis of each cow's change in ELISA results between the initial and repeat samples. The *P* values obtained for multiple comparisons of means were adjusted, by use of the Tukey least-square means method. All statistical analyses were performed by use of a computer program.[d] Results were considered significant at values of $P < 0.05$.

Results

Animals—Between 1999 and 2001, repeat sera were collected from 3,757 adult dairy cattle on 11 dairies in Colorado. Within-herd seroprevalence ranged from 1.2 to 7.8% (mean ± SD, 3.0 ± 2.0%). Size of source dairies ranged from 112 to 2,519 cattle (mean, 793 ± 748 cattle). Interval between repeat samples ranged from 77 to 600 days (mean, 303 ± 77

Table 1—Distribution of cows from which 2 serum samples were obtained for use in testing with an ELISA for detection of antibodies against *Mycobacterium avium* subsp *paratuberculosis* (MAP) on the basis of 6 intervals between repeat samples

Interval	Range (d)	Cows	
		No.	%
1	77–118	177	4.7
2	127–173	249	6.6
3	183–239	415	11.1
4	240–298	618	16.5
5	306–359	2,139	56.9
6	360–600	159	4.2

days). Most (2,139/3,757; 56.9%) cows in this data set had a repeat serum sample collected at an interval of 10 to 12 months after the initial serum sample (**Table 1**). This was attributable to the fact that 4 dairies elected to repeat the whole-herd test approximately 1 year after the initial test.

Proportion of cows in LPH herds that changed ELISA status on the second sample—In LPH herds, only 7 of 23 (30.4%) cows with initial positive results had positive results for the second sample (**Table 2**). Of the cows with initial positive results, 8 (34.8%) became test-suspect and 8 (34.8%) became test-negative for the second sample. The majority (74/104; 71.2%) of cows that initially had test-suspect results had negative results for the second sample, whereas 20 (19.2%) cows again had test-suspect results and 10 (9.6%) cows had positive results for the second sample. Nearly all (976/1,041; 93.8%) of the cows with negative results for the initial sample had negative results for the second sample, whereas 47 (4.5%) had test-suspect results and only 18 (1.7%) had positive results for the second sample.

Proportion of cows in HPH herds that changed ELISA status on the second sample—In HPH herds, 88 of 134 (65.7%) cows with initial positive results also had positive results for the second sample, whereas 29 (21.6%) were test-suspect and only 17 (12.7%) had negative results for the second sample (Table 2). There were 114 of 304 (37.5%) test-suspect cows that maintained suspect status for the second sample, whereas 84 (27.6%) had positive results and 106 (34.9%) had negative results for the second sample. The majority (1,589/2,151; 73.9%) of cows that had initial negative results also had negative results for the second sample, whereas 352 (16.3%) were test-suspect and 210 (9.8%) had positive results for the second sample.

Proportion of all cows that changed ELISA status on the second sample—When results of cows from all herds were combined, 95 of 157 (60.5%) cows that had positive results for the initial sample also had positive results for the second sample, whereas 37 (23.6%) were test-suspects and only 25 (15.9%) had negative results for the second sample (Table 2). Of 408 cows that were initially classified as test-suspects, 134 (32.8%) maintained suspect status for the second sample, whereas 94 (23.0%) had positive results and 180 (44.1%) had negative results for the second sample. The majority (2,565/3,192; 80.4%) of cows with initial negative results had negative results for the second sample, whereas 399 (12.5%) were test-suspects and only 228 (7.1%) had positive results for the second sample.

Comparison of various cutoffs values for classification of cattle—On the basis of the kit-recommended cutoff value, 62 of 157 (39.5%) cows that had positive results on the initial sample (ie, S/P ≥ 0.25) had negative results (ie, S/P < 0.25) for the second sample (**Table 3**). When the cutoff value for a positive result for the initial sample was increased to an S/P ≥ 0.40, 13 of 71 (18.3%) cows had negative results for the second sample. When the cutoff value for a positive result for the initial samples was increased to an S/P ≥ 0.70, only 3 of 33 (9.1%) cows had negative results for the second sample. Most (49/86; 57.0%) cows that had S/P values within the range of 0.25 to 0.39 for the initial sample converted and had negative results for the second sample; however, only 10 of 38 (26.3%) cows that had S/P values within the range of 0.40 to 0.70 for the initial sample converted and had negative results for the second sample.

Comparison of mean number of days between repeat samples and change in ELISA status—Mean number of days between repeat samples was calculated for each of 9 defined categories (**Table 4**). We did not detect a significant interaction between category and within-herd prevalence. Results of an ANCOVA that controlled for within-herd prevalence (HPH or LPH) revealed that the mean number of days between repeat

Table 2—Results of an ELISA for antibodies against MAP for an initial and a second serum sample obtained at variable intervals from cows in herds with low within-herd prevalence (LPH), herds with high within-herd prevalence (HPH), or both

Initial result*	Repeated result*								
	LPH†			HPH			LPH and HPH		
	Positive	Suspect	Negative	Positive	Suspect	Negative	Positive	Suspect	Negative
Positive	7 (30.4)	8 (34.8)	8 (34.8)	88 (65.7)	29 (21.6)	17 (12.7)	95 (60.5)	37 (23.6)	25 (15.9)
Suspect	10 (9.6)	20 (19.2)	74 (71.2)	84 (27.6)	114 (37.5)	106 (34.9)	94 (23.0)	134 (32.8)	180 (44.1)
Negative	18 (1.7)	47 (4.5)	976 (93.8)	210 (9.8)	352 (16.3)	1,589 (73.9)	228 (7.1)	399 (12.5)	2,565 (80.4)

Values reported are number of cows, with percentages reported in parentheses.
*Results were as follows: positive, ratio of the value for the sample to value for the positive-control serum (S/P) ≥ 0.25; suspect, S/P value 0.10 to 0.24; negative, S/P value 0.0 to 0.09. †Sample size for cows originating from LPH herds was relatively small and may not be representative of an actual pattern for changes in ELISA results.
Seroprevalence in LPH herds was < 5%, whereas seroprevalence in HPH herds was ≥ 5%.

Table 3—Proportion of cows that had positive results for an initial sample but negative results for a second sample when the cutoff value for a positive test result of the initial sample was increased from ≥ 0.25 to ≥ 0.40 or ≥ 0.70

Criteria for positive result (S/P value)	No. of cows with positive results on initial sample	Cows that had negative results* on repeated sample	
		No.	%
0.25 to 0.39	86	49	57.0
0.40 to 0.69	38	10	26.3
≥ 0.25	157	62	39.5
≥ 0.40	71	13	18.3
≥ 0.70	33	3	9.1

*Negative results defined as S/P value < 0.25.

Table 4—Number of cows and mean ± SEM number of days between repeated collection of samples for use in an ELISA to detect antibodies against MAP for each of 9 defined categories

Category	Initial sample*	Repeated sample*	No. of cows	No. of days between initial repeat sample
1	Positive	Positive	95	300.4 ± 7.7
2	Positive	Suspect	37	305.2 ± 12.3
3	Positive	Negative	25	313.6 ± 14.5
4	Suspect	Positive	94	290.1 ± 7.9
5	Suspect	Suspect	134	310.0 ± 6.8†
6	Suspect	Negative	180	301.5 ± 5.7
7	Negative	Positive	228	303.0 ± 5.1
8	Negative	Suspect	399	305.2 ± 3.8‡
9	Negative	Negative	2,565	287.2 ± 1.5†‡

*Results were as follows: positive, S/P value ≥ 0.25; suspect, S/P value 0.10 to 0.24; and negative, S/P value 0.0 to 0.09. †,‡Values for pairs of least-squares means differ significantly (†$P = 0.026$; ‡$P < 0.001$).

samples was significantly different between categories 5 and 9 as well as between categories 8 and 9. Overall, mean number of days between samples was similar across all categories of cows, regardless of whether ELISA result changed or remained the same between the initial and repeat samples.

Discussion

Numerous anecdotal reports from the dairy industry indicate the nonrepeatability of ELISA results when the same cow is retested at a later date. Such reports can lead to loss of confidence in the use of diagnostics for this disease. Few studies have provided results of repeated ELISA tests in cattle. Ellis et al[13] reported that many cows originating from LPH herds in Australia changed status when samples were obtained at variable intervals. Fifty-nine percent of cows with a borderline status and 36% of cows with positive results during initial testing with another ELISA[e] had negative results for additional samples (intervals not specified). These findings are similar to results of the study reported here in which 71% of cows in LPH herds that were test-suspects and 35% of cows with positive results for the initial sample had negative results for repeat samples obtained at variable intervals (Table 2).

Cows with negative results were less likely to change ELISA status than cows with positive results or test-suspect cows, regardless of within-herd prevalence of the herd of origin. The HPH herds had a greater proportion of cows that changed from negative to positive ELISA status, compared with LPH herds (10% vs 2%, respectively); however, the sample size for cows originating from LPH herds was relatively small, and the differences in ELISA status that were observed may not have been representative of an actual pattern. Managers of herds with evidence of MAP infection and seroprevalence ≥ 5.0% (ie, HPH herds) should be informed that up to 10% of cows with negative results on the ELISA may seroconvert and have positive results when additional samples are obtained within the intervals reported in this study (77 to 600 days). Seroconversion of specific cattle has been reported by other authors and may be a result of disease progression in chronic syndromes such as MAP infection. In the study reported here, 157 cattle had positive results on the initial S/P, and 417 cattle had positive results on the repeat S/P, indicating that a proportion of the cattle used in this study seroconverted during the mean interval (approx 10 to 12 months).

In 1 study,[15] 28 of 57 (49%) cows from 3 herds that were endemic for MAP infection converted from ELISA-negative status to ELISA-positive status during a 3-year period. Cox et al[15] obtained additional samples from 57 cows and reported that none of the cattle with positive results converted to negative status when repeat samples were obtained during 2- to 6-month intervals for up to 3 years. The majority (56.9%) of samples included in the study reported here were obtained at intervals of 10 to 12 months, which is evident in the mean interval of approximately 10 months for each category of cows (Table 4). An ANCOVA was used to make a decision about whether all cows in this study could be combined into 1 group for reporting of repeat data. Two pairs of categories had least-squares means that were significantly different from each other (5 and 9, 8 and 9), because the mean interval for category 9 (287 days) was the shortest of all categories. Category 9 contained cows that maintained negative status for both samples, which may indicate that a shorter interval between collection of samples could result in fewer cows converting from seronegative to test-suspect or seropositive status. On the basis of results of the ANCOVA and least-squares means analysis, we decided to report the findings from all cows as a single group (LPH and HPH combined) and did not separate the results on the basis of interval between collection of samples.

Test-suspect cows were not likely to have positive results for repeat samples over the range of intervals and the range of herd prevalences reported here. Only 10% of test-suspect cows in LPH herds had positive results for the second sample, and only 28% of test-suspect cows in HPH herds had positive results for the repeat sample. A large proportion of test-suspect cows changed to negative status for the second sample (71% for cows in LPH herds, 35% for cows in HPH herds), which is useful for educating producers about expectations when additional samples are collected from these cattle. Unfortunately, it was not possible to confirm the infection status of cows that were included in the study. Therefore, it is not possible at this time to comment on the likelihood that test-suspect cows were truly infected on the basis of a single ELISA result.

Management decisions for test-suspect cows depend on the aggressiveness of the disease control

program and economic considerations specific to each dairy. The kit manufacturer does not recommend the use of a suspect category, but this category has been used to help minimize the number of cows that fluctuate from negative to positive results when the S/P value is near the cutoff value. The large proportion of test-suspect and seropositive cows that change to seronegative status when repeat samples are tested may indicate that another set of cutoff values would be useful for classification of these cattle in the future.

Increasing the S/P value used to define the cutoff value for classification of seropositive cows resulted in greater predictability of repeat data. However, sensitivity was lost in that fewer seropositive cows were initially identified. When the cutoff value for positive results of an initial S/P was increased from ≥ 0.25 to ≥ 0.40 or ≥ 0.70, cows with results in this range were much more likely to maintain the kit-defined seropositive status (≥ 0.25) for the second sample, versus cows that were classified as seropositive on the basis of an initial S/P ≥ 0.25. These cutoff values may be useful for predicting those seropositive cows that are more likely to remain seropositive for subsequent samples.

[a]IDEXX *Mycobacterium paratuberculosis* antibody test kit, IDEXX Laboratories Inc, Westbrook, Me.

[b]Hirst HL, Garry F, Dinsmore RP, et al. Serologic survey for Johne's disease in Colorado dairies: relationship of seropositivity to herd characteristics (abstr), in *Proceedings*. Int Symp Vet Epidemiol Econ IX 2000;900–902.

[c]Hirst HL. *Johne's disease on Colorado dairies: association of herd characteristics with seroprevalence and behavior of an ELISA when cattle were resampled at two to twenty month intervals*. Master's thesis, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Ft Collins, Colo, 2001.

[d]SAS, version 8.1, SAS Institute Inc, Cary, NC.

[e]ParaChek Johne's absorbed EIA, CSL Ltd, Parkville, Australia.

References

1. Benedictus G, Dijkhuizen A, Stelwagen J. Economic losses due to paratuberculosis in dairy cattle. *Vet Rec* 1987;121:142–146.

2. Buergelt CD, Duncan JR. Age and milk production data of cattle culled from a dairy herd with paratuberculosis. *J Am Vet Med Assoc* 1978;173:478–480.

3. Nordlund KV, Goodger WJ, Pelletier J, et al. Associations between subclinical paratuberculosis and milk production, milk components, and somatic cell counts in dairy herds. *J Am Vet Med Assoc* 1996;208:1872–1876.

4. Ott SL, Wells SJ, Wagner BA. Herd-level economic losses associated with Johne's disease on US dairy operations. *Prev Vet Med* 1999;40:179–192.

5. Sweeney RW, Whitlock RH, Buckley CL, et al. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. *J Vet Diagn Invest* 1995;7:488–493.

6. Johnson-Ifearulundu Y, Kaneene JB, Lloyd JW. Herd-level economic analysis of the impact of paratuberculosis on dairy herds. *J Am Vet Med Assoc* 1999;214:822–825.

7. Johne's Information Center. Spread and control of Johne's disease in a dairy herd (simulation). University of Wisconsin Johne's disease Web site. Available at: www.johnes.org/general/gallery.html. Accessed Feb 2002.

8. Collins MT, Sockett DC. Accuracy and economics of the USDA-licensed enzyme-linked immunosorbent assay for bovine paratuberculosis. *J Am Vet Med Assoc* 1993;203:1456–1463.

9. Dargatz DA, Byrum BA, Barber LK, et al. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. *J Am Vet Med Assoc* 2001;218:1163–1166.

10. Collins MT. Diagnosis of paratuberculosis. *Vet Clin North Am Food Anim Pract* 1996;12:357–371.

11. Whitlock RH, Wells SJ, Sweeney RW, et al. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. *Vet Microbiol* 2000;77:387–398.

12. Bulaga LL. US voluntary Johne's disease herd status program for cattle, in *Proceedings*. US Anim Health Assoc 1998;102:420–433.

13. Ellis TM, Norris RT, Martin PAJ, et al. Evidence for freedom from Johne's disease in cattle and goats in Western Australia. *Aust Vet J* 1998;76:630–633.

14. Collins MT, Angulo A, Buergelt CD, et al. Reproducibility of a commercial enzyme-linked immunosorbent assay for bovine paratuberculosis among eight laboratories. *J Vet Diagn Invest* 1993;5:52–55.

15. Cox JC, Drane DP, Jones SL, et al. Development and evaluation of a rapid absorbed enzyme immunoassay test for the diagnosis of Johne's disease in cattle. *Aust Vet J* 1991;68:157–160.