Effects of positive results for *Mycobacterium avium* subsp *paratuberculosis* as determined by microbial culture of feces or antibody ELISA on results of caudal fold tuberculin test and interferon-γ assay for tuberculosis in cattle

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Objective—To determine whether cattle testing positive for *Mycobacterium avium* subsp *paratuberculosis* as determined by microbial culture of feces or antibody ELISA were more likely to have false-positive responses on the caudal fold tuberculin (CFT) test or interferon-γ (IFN-γ) assay for *Mycobacterium bovis* than cattle testing negative for *M paratuberculosis*.

Animals—1,043 cattle from 10 herds in Michigan.

Procedure—Feces and blood samples for plasma were collected from cattle ≥ 24 months old on the day the CFT test was read. Fecal samples were submitted for microbial culture for *M paratuberculosis*. Plasma samples were tested for antibody against *M paratuberculosis*, and IFN-γ after stimulation with purified protein derivative tuberculin from *M bovis* or *M avium*.

Results—Of 1,043 cattle, 180 (17.3%) had positive CFT test results (suspects) and 8 (0.8%) had positive IFN-γ assay results after stimulation with purified protein derivative tuberculin from *M bovis*. Forty-five (4.3%) and 115 (11.0%) cattle tested positive for *M paratuberculosis* as determined by microbial culture of feces and antibody ELISA, respectively. Cattle with positive responses for *M paratuberculosis* appeared to have an increased likelihood of false-positive results on the CFT test, although this association was not significant.

Conclusions and Clinical Relevance—No significant association was detected among cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces and antibody ELISA and positive CFT test and IFN-γ assay results for *M bovis*. (*J Am Vet Med Assoc* 2005;226:429–435)

Tuberculosis and paratuberculosis are important diseases in cattle caused by *Mycobacterium bovis* and *Mycobacterium avium* subsp *paratuberculosis*, respectively. The **caudal fold tuberculin (CFT)** test and **interferon-γ (IFN-γ)** assay are screening tests for detection of tuberculosis in cattle. Because tuberculosis and paratuberculosis are both caused by mycobacteria, it is believed that cross-reactivity may occur on the CFT test and IFN-γ assay, causing false-positive results in cattle infected with paratuberculosis.[1] The effect of *M paratuberculosis* infection on the CFT test and IFN-γ assay is important for determining confidence of positive tuberculosis test results among herds infected with paratuberculosis.

Tuberculosis in cattle is an important health risk because of the potential spread of the disease to other livestock and humans. Infected cattle often do not have clinical signs; however, some cattle may have chronic weight loss, anorexia, weakness, lethargy, low-grade pyrexia, or a soft, moist, chronic cough. Tuberculosis is presently endemic in free-ranging white-tailed deer (*Odocoileus virginianus*) in northeastern Michigan and has been diagnosed within the same region in 25 beef and 5 dairy cattle herds since 1998.[2,3] Since 2001, tuberculosis has also reemerged in areas of Texas, California, and New Mexico.[4-6]

Intradermal tuberculin tests are the most widely used screening tests for tuberculosis in cattle. The 2 most commonly used intradermal tuberculin tests in cattle in the United States are the CFT test and the **comparative cervical tuberculin (CCT)** test. The CFT test is administered by injecting *M bovis* **purified protein derivative (PPD)** tuberculin into the caudal fold at the base of the tail, whereas the CCT test compares the response from separate injections of *M bovis* and *M avium* PPD tuberculin in the cervical region. Diagnostic tests using PPD tuberculin, however, are limited because most of the proteins in PPD tuberculin are shared among different mycobacteria species.[7] These shared proteins may cause false-positive responses on the CFT test when cattle are exposed to *M paratuberculosis*, *M avium*, *M tuberculosis*, environmental *Mycobacteria* spp, or certain nonmycobacteria agents such as *Nocardia* spp.[8]

The IFN-γ assay is also approved as a screening test for tuberculosis in cattle. This in vitro test measures

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IFN-γ that is released by lymphocytes during a cell-mediated immune response to antigen stimulation. This assay compares the animal's IFN-γ response to *M bovis* and *M avium* PPD tuberculin. In that regard, it is similar to the CCT test that measures the difference in skin swelling caused by inoculation with *M bovis* or *M avium* PPD tuberculin. Advantages of the IFN-γ assay, compared with the CCT test, are that the IFN-γ assay can be performed repeatedly without the need for a rest period for the animal, there is no need to hold cattle for 72 hours and make a second visit to the farm to read the test, and the subjective differences in interpreting the results between veterinarians are eliminated.

In 1981, a study[9] was published in which cattle infected with *M avium* complex serotypes 6, 14, and 18 had reactions that would have been classified as suspect by use of the CFT test. The reclassification of *M paratuberculosis* as a subspecies of *M avium* causes concern that infection with this agent may cause a response similar to that seen with *M avium* on the CFT test.[10]

Paratuberculosis is a chronic disease characterized by thickening of the intestine leading to diarrhea and weight loss despite a normal appetite. Cattle are most susceptible to infection during their first year of life, but clinical signs generally do not develop until the animal is > 2 years old.[11] Estimates of the prevalence of *M paratuberculosis* infection in individual cattle range from 1.6% to 20% in different regions of the United States.[12-16] Results of a study[16] in Michigan dairy herds that used an antibody ELISA to detect cattle with paratuberculosis indicate that 55% of herds had ≥ 2 cows that were seropositive for *M paratuberculosis*, compared with results of another study[17] estimating that nationally, 21.6% of herds are seropositive for *M paratuberculosis*.

The purpose of the study reported here was to determine whether cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces or antibody ELISA were more likely to have false-positive responses on the CFT test or IFN-γ assay than cattle testing negative for *M paratuberculosis*. Evaluating the effect of *M paratuberculosis* on the CFT test and IFN-γ assay in field and laboratory conditions is an important step for determining the amount of confidence given to suspect CFT test results or positive IFN-γ assay results in herds with paratuberculosis.

Materials and Methods

Cattle—Samples of feces and blood were collected from 1,043 Holstein cattle from 10 separate herds in 3 counties in Michigan. Eight of the herds sampled were located in a high-risk area for tuberculosis (Alpena county), 1 herd was located in a tuberculosis-free area (Ingham county), and 1 herd was near the high-risk area (Ogemaw county). Criteria for inclusion in the study included willingness of the farmer to participate in the study, < 250 cattle in the herd (for economic reasons), and permission to sample the herd on the day that the required whole-herd CFT tests were read. All cattle ≥ 24 months old were sampled from each herd. None of the herds had been vaccinated for *M paratuberculosis* or *M bovis*.

CFT and CCT tests for tuberculosis—The CFT tests were performed by accredited veterinarians in private practice or veterinarians employed by the **Michigan Department of Agriculture (MDA)** using methods described in the USDA Uniform Methods and Rules.[18] The same MDA veterinarian tested 7 of the 10 herds. The CCT test was performed on cattle identified as suspect (any visible or palpable response) by the CFT test. All CCT tests were performed by USDA or MDA veterinarians. Results of CFT and CCT tests were interpreted 72 ± 6 hours after cattle were injected with tuberculin. Cattle identified as suspects and reactors by the CCT test were euthanatized, and necropsy was performed at the Diagnostic Center for Population and Animal Health at Michigan State University. Cattle were sedated with xylazine[a] and euthanatized on trailers outside of the laboratory with an overdose of pentobarbital.[b] Microbial culture for *Mycobacterium* spp was performed on 3 sets of pooled tissues. The first pool included sections from parotid, mandibular, and medial retropharyngeal lymph nodes bilaterally. The second pool included sections of cranial and caudal mediastinal and right and left tracheobronchial lymph nodes. The third pool included hepatic, jejunal, and cecal lymph nodes. Histologic examination was performed on the same 3 sets of pooled tissues. If any gross lesions were detected, samples of visceral organs were also obtained for histologic examination and microbial culture.

Sample collection—Fecal samples were collected on the day the CFT test was read. A new plastic sleeve was used for collection of fecal samples from each animal. Fecal samples were placed in separate sterile plastic bags[c] after collection. Fecal samples were transported to the Diagnostic Center for Population and Animal Health at Michigan State University and stored at –80°C until cultured. Blood samples were collected from the first 5 herds on the day the CFT test was administered and again on the day the CFT test was read. Results of antibody ELISA tests for antibodies against *M paratuberculosis* were similar for the 2 collection dates ($\chi^2 = 1.363$; $P = 0.506$); therefore, blood samples from subsequent herds were only collected and analyzed on the day the CFT test was read to conserve resources. The IFN-γ assay was performed with blood sampled on the day the CFT test was read because results of a previous study[19] indicate that higher IFN-γ responses were detected from samples collected 3 days after skin testing. Blood was collected via the middle coccygeal vein by use of a 20-gauge, 1-inch needle and a 10-mL evacuated tube containing sodium heparin.[d] Blood samples were transported to the laboratory in plastic coolers, chilled with ice packs, and processed within 24 hours of sample collection.

Microbial culture of feces and ELISA—Microbial culture of feces for detection of *M paratuberculosis* was performed by use of standard procedures recommended by the USDA National Veterinary Service Laboratory and based on procedures used by Fyock and Whitlock.[20] A commercially available ELISA kit[e] was used to test for antibody against *M paratuberculosis*, as recommended by the manufacturer. Because plasma was required for IFN-γ testing, plasma rather than serum was used for antibody ELISA to avoid sampling each animal twice. Similar results were obtained for antibody ELISA performed with plasma and serum from 1 of the 10 herds that was tested; therefore, use of plasma for antibody ELISA was determined to be acceptable. All samples were tested in duplicate by use of a 24-well plate, and mean **optical density (OD)** was calculated for each pair of wells. Positive and negative control samples from the manufacturer were also tested in duplicate. The corrected OD was calculated by subtracting the mean OD of the 2 negative control wells from the mean OD of duplicate sample wells. A corrected OD > 0.1 was considered as a positive result.

The IFN-γ assay—Blood samples were tested for IFN-γ by use of a commercially available antigen-capture ELISA, following the manufacturers' recommended protocol.[f]

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Heparinized blood from each animal was separated into 4 aliquots of 1.5 mL each and placed into 4 wells of a 24-well plate for each animal. The first well contained 100 µL of phosphate-buffered saline solution (without antigen) as a negative control. The second well contained 100 µL of *M bovis* PPD tuberculin, and the third well contained 100 µL of *M avium* PPD tuberculin to stimulate production and release of IFN-γ. Pokeweed mitogen[g] was added to the fourth well to a final concentration of 10 µg/mL of blood.[21] The pokeweed mitogen was used as a control for cell function because this mitogen stimulates production and release of IFN-γ from mononuclear leukocytes. Plates were incubated for 16 to 24 hours at 38°C in a humidified atmosphere. Plates were centrifuged at 1,200 X *g* for 15 minutes after incubation. Plasma was harvested from each well and stored frozen at –20°C until used. Duplicate wells were used for each sample collected from cattle. By use of the corrected OD results, an animal was considered as positive for *M avium* IFN-γ if the difference between the OD stimulated from *M avium* PPD tuberculin and the OD from the negative control was ≥ 0.1 and the difference between the OD stimulated from *M avium* PPD tuberculin and the OD stimulated from *M bovis* PPD tuberculin was also ≥ 0.1. An animal was considered as having a positive IFN-γ assay result for *M bovis* if the difference between the OD stimulated from *M bovis* PPD tuberculin and the OD from the negative control was ≥ 0.1 and the difference between the OD stimulated from *M bovis* PPD tuberculin and the OD stimulated from *M avium* PPD tuberculin was also ≥ 0.1.

Statistical analyses—A χ^2 test was used to evaluate results between testing for antibody against *M paratuberculosis* on samples obtained the day the CFT test was administered and samples obtained the day the CFT test was read. Prevalence of *M paratuberculosis* within each herd was computed for each type of test (microbial culture of feces or antibody ELISA) by the number of cattle with positive results for each test divided by the total number of cattle tested within each herd. The Spearman rank correlation coefficient was calculated to analyze the correlation between the results from *M paratuberculosis* fecal culture and antibody ELISA.

Additional statistical analyses were performed in 2 parts by use of a standard software package.[h] In the first part, multivariable logistic regression models with random effects were developed to assess associations among CFT test results and *M paratuberculosis* infection status as determined by microbial culture of feces and antibody ELISA, age of animal, and the veterinarian performing the CFT tests. Because the analysis was performed on individual animals, the random effect function was used in all models to adjust for the fact that cattle from the same herd are more alike in terms of exposure to *M paratuberculosis* than cattle in other herds. Model outcome was CFT test result (suspect or negative), and risk factors included veterinarian performing the CFT test, age of animal, results of individual tests for *M paratuberculosis*, and prevalence of *M paratuberculosis* in the herd as determined by each test. Positive IFN-γ responses stimulated by *M avium* were also included as a risk factor because *M paratuberculosis* is considered a subspecies of *M avium*. In the second part, the IFN-γ assay response for tuberculosis was the outcome and risk factors included age of animal, results of individual tests for paratuberculosis, and prevalence of *M paratuberculosis* in the herd as determined by each test.

A backwards-stepwise model development approach was used to generate final models with risk factors significant at $P \leq 0.05$. In brief, a full model was generated, and possible interactions and confounding were assessed and corrected during the model development process. **Odds ratios (ORs)** with 95% confidence intervals were computed for parameter estimates. With the exception of the potential confounders forced into the model, each risk factor was tested by examining the effects of removal of that factor from the model. If removal of the risk factor resulted in a change in the ORs of the remaining variables of > 10%, the risk factor and its interaction terms were retained in the model.

Results

Of the 1,043 cattle tested in this study, 45 (4.3%) had positive results for *M paratuberculosis* as determined by microbial culture of feces and 115 (11.0%) had positive results as determined by antibody ELISA **(Table 1)**. The prevalence of *M paratuberculosis* in each herd as determined by microbial culture of feces and antibody ELISA ranged from 0% to 15.4% (mean, 4.5%; median, 2.8%) and 1.2% to 30.8% (mean, 9.7%; median, 6.9%), respectively **(Figure 1)**.

Of the 1,043 cattle, 180 (17.3%) were identified as suspects by the CFT test. Herd response ranged from 8.0% to 56.1% (mean, 21.2%; median, 20.1%) for the CFT test (Figure 1). Five of the 180 cattle were identified as suspects and a sixth animal was identified as a reactor by the CCT test. Those 6 animals were classified as negative for tuberculosis on the basis of necropsy results and results of histologic examination and microbial culture of tissues. The percentage of cattle with positive CFT test results ranged from 16.6% in cattle testing negative for *M paratuberculosis* as determined by microbial culture of feces and antibody ELISA to 25.0% in cattle testing positive for *M paratuberculosis* as determined by both tests **(Figure 2)**. The difference in positive CFT test results was only 7% between cattle with positive and negative tests results as determined by microbial culture of feces and 4% between cattle with positive and negative tests results as determined by antibody ELISA for *M paratuberculosis*.

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Table 1—Number (%) of cattle testing positive for *Mycobacterium avium* subsp *paratuberculosis* as determined by microbial culture of feces or antibody ELISA and with suspect or positive results for *Mycobacterium bovis* as determined by the caudal fold tuberculin (CFT) test or interferon-γ (IFN-γ) assay, respectively.

Test for *M paratuberculosis*	All cattle (n = 1,043)	CFT suspect (180)	CFT negative (863)	IFN-γ positive (8)
Microbial culture of feces	45 (4.3)	11 (6.1)	34 (3.9)	0
Antibody ELISA	115 (11.0)	24 (13.3)	91 (10.5)	0
Microbial culture of feces or antibody ELISA	144 (13.8)	31 (17.2)	113 (13.1)	0
Microbial culture of feces and antibody ELISA	16 (1.5)	4 (2.2)	12 (1.4)	0
Negative results for both tests	899 (86.2)	149 (82.8)	750 (86.9)	8 (100)

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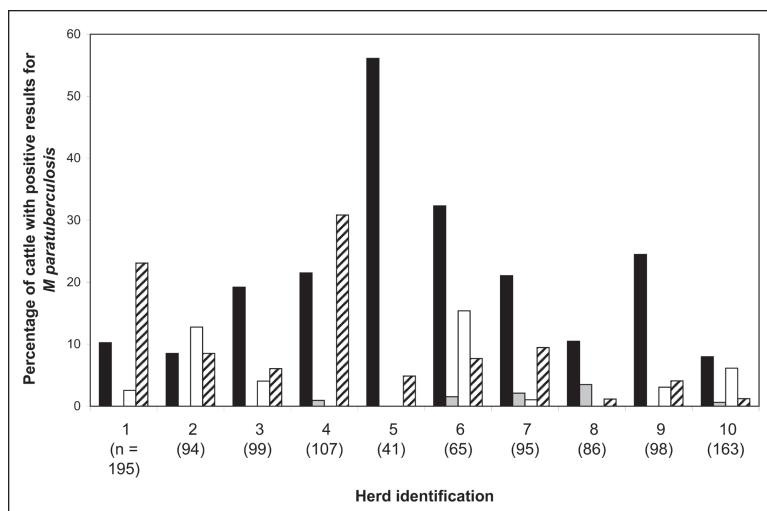

Figure 1—Percentage of cattle in each of 10 testing positive for *Mycobacterium avium* subsp *paratuberculosis* as determined by microbial culture of feces (white bar) or antibody ELISA (striped bar) and with suspect or positive results for *Mycobacterium bovis* as determined by caudal fold tuberculin (CFT) test (black bar) or interferon-γ (IFN-γ) assay (shaded bar), respectively.

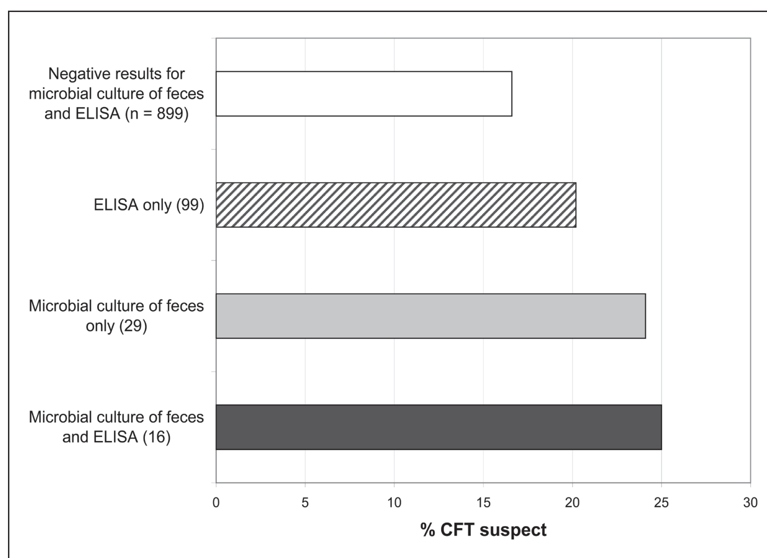

Figure 2—Percentage of cattle with positive CFT test results (suspect) determined on the basis of cattle with positive test results for *M paratuberculosis* as determined by microbial culture of feces, antibody ELISA, or both.

Eight cattle (0.8%) had positive IFN-γ assay results after stimulation of blood samples with *M bovis* PPD tuberculin. Herd response ranged from 0.0% to 3.5% (mean, 0.9%; median, 0.3%; Figure 1). Two of the 8 cattle with positive IFN-γ assay results were identified as suspects by the CFT test; however, both animals had negative CCT test results. Because this study was performed before the IFN-γ assay became an official screening test for tuberculosis, none of the 8 cattle that had positive IFN-γ assay results were removed from the herd for postmortem examination. In addition, none of those 8 cattle tested positive for *M paratuberculosis* as determined by microbial culture of feces or antibody ELISA. *Mycobacterium bovis* was not diagnosed on any of the farms in this study.

In the first part of the statistical analyses, IFN-γ released from the stimulation of blood with *M avium* PPD tuberculin was the only significant risk factor associated with a positive CFT test (OR = 5.4; $P < 0.001$; **Table 2**). Cattle with positive results for *M paratuberculosis* as determined by microbial culture of feces or antibody ELISA appeared to have an increased likelihood of false-positive results on the CFT test, although the association was not significant.

The veterinarian performing the CFT test was identified as a confounding variable and therefore controlled for in the modeling process. Results of microbial culture of feces for *M paratuberculosis* and antibody ELISA were poorly correlated with a Spearman rank correlation coefficient of 0.1663 ($P < 0.001$).

The IFN-γ assay results after stimulation with *M bovis* PPD tuberculin for each animal were analyzed with results of microbial culture of feces and antibody ELISA for individual cattle, prevalence of paratuberculosis in each herd as determined by each test, and age of animal. None of the cattle with positive IFN-γ assay results also tested

Table 2—Results of a multivariable logistic regression analysis of the effects of positive test results for *M paratuberculosis* as determined by microbial culture of feces or antibody ELISA separately on results of CFT test (classified as suspect or negative) for tuberculosis in cattle.

	Final model				
Risk factor	**Estimate**	**SE**	**χ^2 *P* value**	**OR**	**95% CI**
M avium IFN-γ	1.6955	0.404	< 0.0001	5.45	2.47–12.03
Age (mo)	0.0084	0.005	0.0695	1.01	0.999–1.02
Veterinarian performing CFT test					
1	–2.0731	1.22	0.0904	0.13	0.011–1.38
2	–0.9467	1.21	0.4331	0.39	0.036–4.13
3	0.1268	0.89	0.8866	1.13	0.199–6.49
4	0	NA	NA	1.00	NA

OR = Odds ratio. CI = Confidence interval. NA = Not applicable.

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positive for *M paratuberculosis* as determined by antibody ELISA or microbial culture of feces. No variables were significantly associated with positive IFN-γ assay results after stimulation with *M bovis* PPD tuberculin.

Discussion

Cross-reactivity between different mycobacterial antigens has led to the suggestion that infection with *M paratuberculosis* may result in false-positive responses on the CFT test.[1] Determination of the cause of false-positive responses is important because they may lead to unnecessary culling of cattle, increased time and costs associated with handling of cattle, increased cost of follow-up testing, and psychologic stress to producers and veterinarians.

Results of a study[22] indicate that false-positive responses on the CFT test were detected after vaccination of calves against *M paratuberculosis*. In that study, 4 calves were vaccinated at 28 days of age with a commercially available modified-live *M paratuberculosis* vaccine, resulting in positive or suspicious skin reactions on the CFT test for as long as 2 years after vaccination. Vaccination caused a cell-mediated immune reaction detectable by intradermal tests for tuberculosis for a long duration. In the study reported here, we evaluated the association between natural *M paratuberculosis* infection as determined by microbial culture of feces and antibody ELISA and results of the CFT test; therefore, adult cattle that had not been vaccinated for *M paratuberculosis* were used.

Paratuberculosis infection status is extremely difficult to accurately determine in individual cattle. Use of microbial culture of feces for *M paratuberculosis* as a measure of true infection would considerably underestimate the number of infected cattle in a herd because the sensitivity of the test ranges from 45.1% to 87.7%, depending on the definition of infection.[23,24] Therefore, we used results of tests for paratuberculosis, rather than infection status, to evaluate the association of paratuberculosis with positive test results for tuberculosis. Because herds were chosen on the basis of the date of CFT testing for tuberculosis and not on paratuberculosis infection status, likelihood ratios could not be generated. Herds could have been chosen on the basis of paratuberculosis infection status and then voluntarily tested for tuberculosis for the purposes of this study; however, detection of cattle with positive tuberculosis test results would have caused severe regulatory problems. In the study reported here, we attempted to evaluate the closest measurement of true paratuberculosis infection; therefore, the multiple logistic regression models were used to test the associations among cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces or antibody ELISA considered separately and cattle with positive CFT test results. Associations among cattle testing positive for *M paratuberculosis* as determined by both microbial culture of feces and antibody ELISA and cattle with positive CFT test results were also tested.

A higher percentage of cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces, antibody ELISA, and a combination of those 2 tests had positive CFT test results, compared with cattle testing negative for *M paratuberculosis* as determined by microbial culture of feces and antibody ELISA. The most unexpected finding was the lack of a significant association at either the individual animal or herd level between positive test results for paratuberculosis and risk of CFT test response. There may have been several reasons for the lack of a significant association between positive test results for paratuberculosis and CFT test results. First, both tests used to detect *M paratuberculosis* have low sensitivities. In addition to the low sensitivity of fecal culture reported above, the sensitivity of the antibody ELISA has been shown to be 31.1% in cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces and as much as 47.3% in cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces or internal organs.[25,26] A low sensitivity would result in a high number of false-negative results, causing an underestimation of the true number of cattle infected with *M paratuberculosis*. This misclassification would reduce the power of the tests in this study to detect a true positive association between cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces or antibody ELISA and a positive CFT test result. Second, the sample size may have been too small to detect a true association. In the study reported here, the sample size was determined by the ability to detect a 25% difference in positive CFT test results among cattle testing positive and negative for *M paratuberculosis* as determined by microbial culture of feces or antibody ELISA. The differences in positive CFT test results were lower than expected with only a 7% difference between cattle with positive and negative test results by microbial culture of feces and 4% between cattle with positive and negative tests results by antibody ELISA for *M paratuberculosis*. With 80% power (the probability of detecting a true association), the true sample size required to detect a significant association between *M paratuberculosis* tests and the CFT test would have to be 6,304 as determined by microbial culture of feces and 7,736 as determined by antibody ELISA, compared with the total sample size of 1,043 used for this study. Third, the true prevalence of paratuberculosis may have been low among all herds tested for this study. There were seemingly no herds with a high prevalence of paratuberculosis, which would have helped to increase the sample size of cattle with positive test results for *M paratuberculosis* and increased the power of detecting an association among positive test results for *M paratuberculosis* and positive CFT test results at the herd level. Fourth, it is possible that cross-reactivity cannot be accurately detected between different branches of the immune system, such as comparing the humoral response to *M paratuberculosis* as detected by antibody ELISA with the cell-mediated response as detected by CFT tests. However, the methodology of all tests used in our study was based on whole organism antigen, which should permit some cross-reactivity.

A combination of these factors may have resulted in the lack of a significant association between positive test results for *M paratuberculosis* and CFT test results. The most likely combination is a misclassification of

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cattle with true positive results as negative for *M paratuberculosis* because of the low sensitivity of the tests that are presently available. This misclassification may have resulted in a difference in the percentage of cattle with positive CFT results among cattle testing positive and negative for *M paratuberculosis* that was smaller than the true difference. This small difference would decrease the power of the test; therefore, a sample size larger than that used in our study would be required to detect a significant association.

The sample size used in this study would have detected an association with a difference of ≥ 25% of positive CFT test results among cattle with positive and negative test results for *M paratuberculosis*. This study was successful in determining that a difference this large between those with positive and negative test results for *M paratuberculosis* did not exist. The higher percentage of positive CFT test results among cattle testing positive for *M paratuberculosis* as determined by microbial culture of feces, antibody ELISA, and a combination of those 2 tests, compared with cattle testing negative for *M paratuberculosis*, may have been attributable to chance alone.

In the study reported here, only 8 of 1,043 cattle tested had positive IFN-γ assay results for tuberculosis. Two of those 8 cattle had positive CFT test results; however, results of the follow-up CCT tests were negative. None of the 8 cattle positive for *M bovis* IFN-γ also tested positive for *M paratuberculosis* as determined by antibody ELISA or microbial culture of feces.

Although no significant association was detected among test results for *M paratuberculosis* and positive *M bovis* IFN-γ results, the lack of an association was only determined by 8 IFN-γ–positive animals. Because of the small number of positive results in the IFN-γ test, a true association caused by *M paratuberculosis* would have been difficult to identify. The sensitivity of the IFN-γ assay used in this study reportedly ranges from 81.8% to 91.7%, and specificity ranges from 84.3% to 99.1%.[27-30] The small number of cattle positive for *M bovis* IFN-γ is in agreement with those findings indicating that the IFN-γ assay has a high specificity for detection of *M bovis*. If *M paratuberculosis* infection does not affect the outcome of the IFN-γ assay, the low number of false-positive results may make the IFN-γ assay a useful supplemental screening test for *M bovis* in herds with paratuberculosis.

The CFT and IFN-γ tests are prone to false-positive results from cross-reactivity because many of the proteins in PPD tuberculin are shared by different mycobacteria species. The recent mapping of the complete genome sequence for *M bovis* permits specific proteins to be identified that are unique to *M bovis*.[31] Results of a recent study[7] indicate that the production of IFN-γ responses from an assay by use of a mixture of proteins ESAT-6, CFP-10, TB27.4, and TB10.4 may have a sensitivity similar to the CFT test but with improved specificity. Further research with a sample size larger than this study is important to determine whether there is an association among cattle testing positive for *M paratuberculosis* and positive CFT test and IFN-γ assay results stimulated by PPD tuberculin or newly identified more specific proteins for *M bovis*.

a. Rompun, Bayer AG, Leverkusen, Germany.
b. Fatal-Plus, Vortach Pharmaceuticals, Dearborn, Mich.
c. Whirl-pak, NASCO, Fort Atkinson, Wis.
d. Corning Glass Works, Corning, NY.
e. Parachek, BioCor Animal Health, Omaha, Neb.
f. Bovigam, BioCor Animal Health, Omaha, Neb.
g. Phytolacca americana, cell culture tested, Sigma Chemical Co, St Louis, Mo.
h. SAS, version 8, SAS Institute Inc, Cary, NC.

References

1. Morrison WI, Bourne FJ, Cox DR, et al. Pathogenesis and diagnosis of infections with *Mycobacterium bovis* in cattle. Independent scientific group on cattle TB. *Vet Rec* 2000;146: 236–242.
2. Schmitt SM, O'Brien DJ, Bruning-Fann CS, et al. Bovine tuberculosis in Michigan wildlife and livestock. *Ann N Y Acad Sci* 2002;969:262–268.
3. Michigan bovine tuberculosis Web site. Maps: TB positive animals. Available at: www.bovinetb.com. Accessed Sep 24, 2003.
4. It's official. Texas loses cattle tuberculosis "free" status [press release]. Austin, Tex: Texas Animal Health Commission, Jun 7, 2002.
5. Bovine tuberculosis found in cattle [press release]. Sacramento, Calif: California Department of Food and Agriculture, Jun 3, 2002.
6. USDA amends bovine tuberculosis classification of New Mexico [press release]. Washington, DC: Animal and Plant Health Inspection Service, Jul 24, 2003.
7. Aagaard C, Govaerts M, Meng Okkels L, et al. Genomic approach to identification of *Mycobacterium bovis* diagnostic antigens in cattle. *J Clin Microbiol* 2003;41:3719–3728.
8. Karlson AG. Nonspecific or cross-sensitivity reactions to tuberculin in cattle. *Adv Vet Sci* 1962;7:148–175.
9. Ketterer PJ, Rogers RJ, Donald B. Pathology and tuberculin sensitivity in cattle inoculated with *Mycobacterium avium* complex serotypes 6, 14, and 18. *Aust Vet J* 1981;57:61–65.
10. McIntyre G, Stanford JL. Immunodiffusion analysis shows that *Mycobacterium paratuberculosis* and other mycobactin-dependent mycobacteria are variants of *Mycobacterium avium*. *J Appl Bacteriol* 1986;61:295–298.
11. Smyth RH, Christie GJ. Some observations on Johne's disease with a further note on the examination of fecal samples. *Vet Rec* 1950;62:429–450.
12. Braun RK, Buergelt CD, Litell RC, et al. Use of an enzyme-linked immunosorbent assay to estimate prevalence of paratuberculosis in cattle of Florida. *J Am Vet Med Assoc* 1990;196:1251–1254.
13. Whipple DL, Callihan DR, Jarnagin JL. Cultivation of *Mycobacterium paratuberculosis* from bovine fecal specimens and a suggested standardized procedure. *J Vet Diagn Invest* 1991;3: 368–373.
14. Thoen CO, Baum KH. Current knowledge on paratuberculosis. *J Am Vet Med Assoc* 1988;192:1609–1611.
15. Kreeger JM. Ruminant paratuberculosis—a century of progress and frustration. *J Vet Diagn Invest* 1991;3:373–382.
16. Johnson-Ifearulundu Y, Kaneene JB. Distribution and environmental risk factors for paratuberculosis in dairy cattle herds in Michigan. *Am J Vet Res* 1990;60:589–596.
17. National Animal Health Monitoring System. Johne's disease on US dairy operations. #N245.1097. Fort Collins, Colo: USDA, Animal and Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health, National Animal Health Monitoring System, 1997.
18. USDA. Bovine tuberculosis eradication. uniform methods and rules, effective January 22, 1999. USDA-APHIS 91-45-011. Riverdale, Md: USDA, 1999.
19. Whipple DL, Palmer MV, Slaughter RE, et al. Comparison of purified protein derivatives and effect of skin testing on results of a commercial gamma interferon assay for diagnosis of tuberculosis in cattle. *J Vet Diagn Invest* 2001;13:117–122.
20. Fyock TL, Whitlock RH. Recommended culture protocol for *Mycobacterium paratuberculosis*. Standard operating procedure. Kennett Square, Pa: New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 1999.

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21. Stabel JR. Production of γ-interferon by peripheral blood mononuclear cells: an important diagnostic tool for detection of subclinical paratuberculosis. *J Vet Diagn Invest* 1996;8:345–350.

22. Köhler H, Gyra H, Zimmer K, et al. Immune reactions in cattle after immunization with a *Mycobacterium paratuberculosis* vaccine and implications for the diagnosis of *M. paratuberculosis* and *M. bovis* infections. *J Vet Med B Infect Dis Vet Public Health* 2001;48:185–195.

23. Sockett DC, Carr DJ, Collins MT. Evaluation of conventional and radiometric fecal culture and a commercial DNA probe for diagnosis of *Mycobacterium paratuberculosis* infections in cattle. *Can J Vet Res* 1992;56:148–153.

24. Zimmer K, Drager KG, Klawonn W, et al. Contribution to the diagnosis of Johne's disease in cattle. Comparative studies on the validity of Ziehl-Neelsen staining, faecal culture and a commercially available DNA-probe test in detecting *Mycobacterium paratuberculosis* in faeces from cattle. *J Vet Med B Infect Dis Vet Public Health* 1999;46:137–140.

25. Reichel MP, Kittelberger R, Penrose ME, et al. Comparison of serological tests and faecal culture for the detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle and analysis of the antigens involved. *Vet Microbiol* 1999;66:135–150.

26. Collins MT, Sockett DC, Ridge S, et al. Evaluation of a commercial enzyme-linked immunosorbent assay for Johne's disease. *J Clin Microbiol* 1991;29:272–276.

27. Lauzi S, Pasotto D, Amadori M, et al. Evaluation of the specificity of the γ-interferon test in Italian bovine tuberculosis-free herds. *Vet J* 2000;160:17–24.

28. Ryan TJ, Buddle BM, DeLisle GW. An evaluation of the gamma interferon test for detecting bovine tuberculosis in cattle 8 to 28 days after tuberculin skin testing. *Res Vet Sci* 2000;69:57–61.

29. Scacchia M, Lelli R, Petrini A, et al. Use of innovative methods in the eradication of bovine tuberculosis. *J Vet Med B Infect Dis Vet Public Health* 2000;47:321–327.

30. Wood PR, Corner LA, Rothel JS, et al. A field evaluation of serological and cellular diagnostic tests for bovine tuberculosis. *Vet Microbiol* 1992;31:71–79.

31. Garnier T, Eiglmeier K, Camus JC, et al. The complete genome sequence of *Mycobacterium bovis*. *Proc Natl Acad Sci U S A* 2003;100:7877–7882.

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