

Relationships Between Fecal Culture, ELISA, and Bulk Tank Milk Test Results for Johne's Disease in US Dairy Herds

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ABSTRACT

Objectives were to estimate percentages of seropositive herds with cows shedding *Mycobacterium paratuberculosis* in feces and milk, and to estimate sensitivity, specificity, and predictive value of an ELISA relative to fecal culture. Dairy cows (n = 712) were randomly selected from 61 herds previously identified by ELISA as positive for Johne's disease. Fecal and bulk tank milk samples (n = 52 of 61 herds) were obtained from 10 states in the United States. Fecal samples were processed by a double centrifugation, double decontamination culture procedure. Milk samples were processed for both culture and DNA analysis by using polymerase chain reaction (PCR). Of 24 herds with at least three cows that had tested ELISA-positive, 79% were also culture-positive, compared with 18 of 37 herds with one or two ELISA-positive cows. Both fecal-culture and ELISA results were available on 651 cows; only 25% of cows that were fecal-culture positive also tested positive by ELISA and over 6% of cows that were fecal-culture negative tested ELISA-positive. Milk samples all cultured negative, but analysis of milk samples by PCR resulted in 68% of herds positive for *M. paratuberculosis* DNA including 24 of 31 herds with positive fecal cultures and 11 of 21 herds with negative fecal cultures. Sensitivity and specificity of the ELISA compared with fecal culture is lower than previously reported and perhaps best used in screening herds because of limited efficacy to predict infection in individual cows. In addition, contamination of bulk tank milk samples with *M. paratuberculosis* does occur in seropositive herds, even in some with negative fecal cultures.

(Key words: Johne's disease, dairy cows, diagnostics)

Abbreviation key: CFU = colony forming unit, NAHMS = National Animal Health Monitoring System, PCR = polymerase chain reaction, PPV = positive predictive value.

INTRODUCTION

Johne's disease in cattle is characterized by a chronic granulomatous enteritis caused by the acid-fast bacterium, *Mycobacterium paratuberculosis* (Chiodini et al., 1984). Typically, cattle become infected as young calves via fecal-oral transmission of the organism and may remain subclinically infected for long periods of time, shedding low numbers of organisms before progression to the terminal stage of infection. Clinical disease is characterized by profuse, nontreatable diarrhea with a high level of bacterial shedding, emaciation, and eventual death. Major economic losses are incurred each year to the dairy and beef cattle industry because of decreased milk production, premature culling of infected cattle, and an increased incidence of mastitis and reproductive disorders (Abbas et al., 1983; Benedictus et al., 1987; Johnson-Ifearulundu and Kaneene, 1997). A recent National Animal Health Monitoring System (NAHMS) survey conducted by the Centers for Epidemiology and Animal Health demonstrated that between 20 and 40% of dairy herds in the United States have some level of Johne's disease in the herd (Wells and Wagner, 2000; Wells et al., 1998). It is estimated that Johne's disease costs the dairy producer between \$45 and \$235 per cow in the herd, depending on the incidence of clinical disease in the herd (Ott et al., 1999).

Diagnosis of Johne's disease presents a major problem in control of this disease. Serologic assays such as ELISA, agar gel immunodiffusion assay, and complement-fixation test, all of which rely on the presence

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of antibodies to *M. paratuberculosis* in the serum, are commonly used to diagnose paratuberculosis in a herd because of the low cost of the test and the rapid availability of test results within 2 to 3 d (Colgrove et al., 1989). The ELISA test is the most sensitive of the three serologic tests currently available on the commercial market, with a reported average sensitivity of 45% but a specificity of 99% or greater (Collins and Sockett, 1993). However, because infected animals generally do not produce measurable antibody titers until the later stages of disease, these tests are relatively ineffective in detecting subclinical infection in cows. Reported sensitivity values for a commercial ELISA kit to detect paratuberculosis antibodies in the sera of cattle ranged from 15 to 57% for subclinically infected cattle, with an average of 88% sensitivity reported for clinically infected cattle, relative to fecal shedding of *M. paratuberculosis* (Collins and Sockett, 1993; Sweeney et al., 1995).

Currently, cultivation of *M. paratuberculosis* from fecal or tissue specimens remains the most definitive method for detecting animals with Johne's disease (Stabel, 1996). The procedure requires 8 to 16 wk of incubation and specialized medium for propagation of the organism. In addition, contamination of culture by overgrowth of competing bacterial and fungal agents is often a problem when culturing *M. paratuberculosis* from fecal specimens. Because most cattle with Johne's disease typically shed the microorganism intermittently in their feces and in low numbers, particularly in the early stages of disease, the sensitivity of fecal culture for properly diagnosing this disease in infected cows is estimated at 45 to 50% (Sockett et al., 1992). Despite these disadvantages, fecal culture is still considered the most sensitive and reliable test for the detection of subclinically infected animals and remains the "gold standard" diagnostic test for Johne's disease in research and diagnostic laboratories in this country (Chiodini et al., 1984; Cocito et al., 1994).

The new epidemiologic data from the NAHMS Dairy 96 Study demonstrated a significant prevalence of Johne's disease in dairy herds throughout the United States. Major efforts are under way to educate producers and veterinarians about this costly disease and to help them develop control and herd health management strategies. Many states are currently implementing their own voluntary Johne's disease control program. Adequate diagnostic tests are an essential element of a control program for any disease. Currently, the National Johne's Working Group, a group of representatives from industry, government, and academe, recommends the combined use of the ELISA and fecal culture to control the spread of this disease between herds. With this information in mind, an add-on study to the

NAHMS Dairy 96 Study was initiated to evaluate the relationship between the ELISA and fecal culture for diagnosis of Johne's disease in dairy herds in different regions of the United States.

MATERIALS AND METHODS

Herds

Fecal and bulk tank milk samples were initially obtained from 79 dairy herds from 10 states (IA, MN, NY, OR, TN, TX, VT, WA, WI, and CA) within the United States. Herds were selected for fecal culture based on their serologic test-positive status from the NAHMS Dairy 96 Study, with at least one cow ELISA-positive within the herd (Wells and Wagner, 2000). The NAHMS Dairy 96 Study tested 31,864 cows from 971 herds in 20 states in the United States by ELISA, thus providing a significant database for selection of seropositive herds. State veterinarians who elected to participate in the add-on study (10 of 20 states) randomly selected an average of eight Johne's disease-positive herds from their state to provide fecal and milk samples for further analyses. Herds were statistically sampled according to herd size as follows: 30 samples, 30 to 49 cows in herd; 40 samples, 50 to 99 cows in herd; 45 samples, 100 to 299 cows in herd; and 50 samples, 300 or more cows in herd. Cows were selected to represent the distribution of cattle within the herd, i.e., lactating, nonlactating, healthy, and sick.

After performing the fecal-culture analysis, the number of herds included in the study was reduced from the original 79 herds to 69 because of 10 herds with heavy contamination of fecal samples with fungi and other nonpathogenic bacteria. The contamination of fecal cultures precluded an accurate reading of representative colony forming units (CFU) on the agar slants. In addition, eight of the 79 herds selected for the add-on study were found to be ELISA-negative because of significant culling of animals from these herds that had previously tested positive during the NAHMS Dairy 96 Study. These herds were also dropped from the data analysis in this study, which resulted in 61 remaining herds representing 10 states.

Samples

Samples were collected within 6 mo of the completion of sampling for the NAHMS Dairy 96 Study. Fecal samples (approximately 20 g) were obtained from 712 individual cows within the 61 selected herds. Feces were collected from each animal rectally by using a clean, dry examination glove. Fecal samples were placed into clean, plastic storage containers with flip-top lids (Polycan, Garwood, NJ), then shipped in polystyrene contain-

ers with ice packs to the National Animal Disease Center via overnight mail. Three bulk tank milk samples representing three different milkings from each of 52 of the 61 herds were collected into separate sterile 50-ml conical tubes (Falcon Blue Max Tubes; Becton-Dickinson, Franklin Lakes, NJ) over a 6-wk period (2 wk between samples) and shipped frozen within the same polystyrene container. Upon receipt, the fecal and milk samples were stored at -70°C until they were processed.

Fecal-Culture Method

Fecal samples (2 g) were added to 35 ml of sterile deionized water, shaken, and allowed to settle at room temperature for 30 min each. After settling, the entire supernatant fraction (25 to 30 ml) was removed and placed in a clean 50-ml tube. Samples were then centrifuged at $1700 \times g$ for 20 min, the supernatant was decanted, and the pellet was resuspended in 30 ml of 0.9% hexadecylpyridinium chloride/brain-heart infusion broth (Sigma Chemical Co., St. Louis, MO, and Difco, Detroit, MI, respectively) solution. After overnight decontamination at 37°C , samples were centrifuged at $1700 \times g$ for 20 min. After supernatants were removed, pellets were resuspended in 1 ml of antibiotic solution (100 $\mu\text{g/ml}$ naladixic acid, 100 $\mu\text{g/ml}$ vancomycin, and 50 $\mu\text{g/ml}$ amphotericin B; Sigma Chemical Co.) and incubated overnight at 37°C . Sample suspensions (0.2 ml) were inoculated onto four tubes of Herrold's Egg Yolk Medium containing naladixic acid (50 $\mu\text{g/ml}$), vancomycin (50 $\mu\text{g/ml}$), and 2 mg/L of mycobactin J (Allied Monitor, Fayette, MO). Inoculated tubes were incubated at 37°C in a horizontal position for 1 wk with loose caps to permit evaporation of residual moisture on the surface of the medium. Caps were then tightened, tubes were returned to an upright position, and incubation of samples continued for a total of 12 wk. Tubes were examined at 4-wk intervals and colonies were enumerated by using a dissecting microscope at $25\times$ magnification.

Processing of Milk Samples

A composite milk sample comprised of 10 ml of milk from each of the three separate milk samples taken from each bulk tank was also cultured for viable *M. paratuberculosis*. Each 30-ml composite milk sample was centrifuged at 2000 rpm for 30 min at room temperature. The supernatant (whey) was removed and the pellet resuspended in 30 ml of 0.9% hexadecylpyridinium chloride/brain-heart infusion broth solution and decontaminated overnight at 37°C . Samples were then centrifuged at 2500 rpm for 20 min and the pellets resuspended in 1 ml of antibiotic solution. Sample sus-

pensions (0.1 ml) were inoculated onto four tubes of Herrold's Egg Yolk Medium containing naladixic acid, vancomycin, and mycobactin J as above. Tubes were incubated at 37°C as per the fecal-culture protocol previously described; however, tubes were examined every 4 wk for a total of 12 wk and colonies enumerated, followed by further incubation for 12 mo after inoculation to allow for appearance of colonies.

For crude extraction of *M. paratuberculosis* DNA from bulk tank milk samples, 0.5 ml of composite milk samples from each herd was added to 1.5-ml Eppendorf tubes. Tubes were centrifuged at 15,000 rpm for 5 min and supernatants were discarded. To each cell pellet, 1 mg of proteinase K (Sigma Chemical Co.) was added and samples were vortex mixed vigorously to resuspend pellets. Samples were incubated overnight at 50°C in a shaking water bath. To each sample, 22 μl of TEN buffer (0.1 M NaCl; 10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0; Sigma Chemical Co.) and 10 μl of 0.4 M NaOH was added and samples were vortex mixed. Tubes were then placed in a boiling water bath for 30 min. Samples were extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1; Amresco, Solon, OH) and vortex mixed, followed by immediate centrifugation at 10,000 rpm for 5 min. The aqueous layer was transferred to a new tube and the DNA precipitated by adding 2.2 volumes of cold 100% ethanol and 0.1 volumes of 3 M sodium acetate (pH 5.2). Samples were mixed gently and put in a -20°C freezer for at least 1 h. Tubes were centrifuged at 15,000 rpm for 15 min and supernatants were aspirated off. Pellets of DNA were air-dried for 2 to 5 min and then pellets were resuspended in 10 μl of sterile water. The DNA was either used immediately or frozen at -20°C for later analysis.

The DNA isolated from milk samples was amplified by using a nested polymerase chain reaction (PCR) protocol as described (Gwodz et al., 1997). For the first amplification reaction, forward and backward primers (5'-GTTCCGGGGCCGTCGCTTAGG-3'; 5'-GAGGTCGATCGCCACGTGA-3') to amplify a 400-bp region of the insertion element, IS900, which is specific for *M. paratuberculosis*. A second amplification reaction further amplified the PCR product above by using internal forward and backward primers (5'-GCTTAGGCTTCGAATTGCC-3'; 5'-CTCCGTAACCGTCATTGTCC-3') and resulted in a final product of 194 bp. After amplification, DNA was electrophoresed in a 4% agarose gel (Reliant Gel Systems, FMC Bioproducts, Rockland, ME) containing ethidium bromide in TBE buffer (89 mM Tris, 89 mM boric acid, and 2 mM EDTA) and bands were visualized by using a UV transilluminator (UV Transilluminator, Bio-Rad, Hercules, CA). A positive control DNA sample (*M. paratuberculosis* DNA)

Table 1. Distribution of ELISA-positive animals in 61 selected and 362 nonselected herds in the add-on study.¹

Herd status	Number of ELISA-positive cows per herd ²			
	1+	2+	≥3+	Total
Nonselected	202 ³ (55.8%)	94 (26%)	66 (18.2%)	362 (100%)
Selected	26 (42.6%)	11 (18%)	24 (39.4%)	61 (100%)
Total	228	105	90	423

¹Sixty-one herds were selected for fecal culture from 423 herds in the NAHMS Dairy 96 Study that had at least one ELISA-positive cow.

²1+ = One positive animal, 2+ = two positive animals, 3+ = three or more positive animals. χ^2 , 2 df = 13.942; $P < 0.01$.

³Number of herds.

was included in each PCR run and on each gel for assay verification. A negative control consisting of buffer only was run each time to verify lack of cross-contamination of samples. Preliminary experiments were conducted with raw milk experimentally inoculated with serial dilutions (10^8 to 10^1) of *M. paratuberculosis* to evaluate the sensitivity of the DNA-PCR protocol. Positive results were repeatedly observed in milk samples spiked with 10^2 *M. paratuberculosis* or more per milliliter of milk but only rarely in samples spiked with 10^1 microorganisms.

Statistical Analyses

To compare the representativeness of herds and cows selected, nonresponse analyses was performed by using χ^2 tests of association (InStat; GraphPad Software, San Diego, CA). Additional χ^2 tests were also run to compare ELISA-positive cows with fecal-culture status and to determine the distribution of fecal-culture-positive cows within each group of ELISA-positive cows. A comparison between herd fecal shedding and bulk tank milk samples was also performed.

RESULTS

The distribution of ELISA-positive animals within nonselected and selected herds for the add-on study is shown in Table 1. Of the 423 ELISA-positive herds identified in the NAHMS Dairy 96 Study, 228 (54%) had one ELISA-positive cow in the herd, 105 (25%) herds had two test-positive cows, and 90 (21%) had three or more ELISA-positive animals. The percentage of herds with one or two cows that tested ELISA-positive was lower for selected than for nonselected herds in this study (56 vs. 43%; 26 vs. 18%); however, the number of herds that had three or more ELISA-positive cows was higher for selected herds (39%) compared with nonselected herds (18%). The herd-level cull rates for cows with clinical signs consistent with Johne's disease

Table 2. Comparison of ELISA-positive cows with fecal-culture status in 61 selected herds.

Fecal culture	Number of ELISA-positive cows per herd ¹			
	1+	2+	≥3+	Total
Positive	13 ² (50%)	5 (45.5%)	19 (79.2%)	37 (60.7%)
Negative	13 (50%)	6 (54.5%)	5 (20.8%)	24 (39.3%)
Total	26	11	24	61

¹1+ = One positive animal, 2+ = two positive animals, 3+ = three or more positive animals. χ^2 , 2 df = 5.75; $P = 0.0565$.

²Number of herds.

for the year previous to this survey were strikingly similar for nonselected and selected herds. Approximately 75% of selected and nonselected herds had reported fewer than 5% of cows culled with clinical signs of Johne's disease in the previous year; whereas 25% of all herds had a ≥5% culling rate because of Johne's disease in the previous year (data not shown).

Selected herds were categorized as to the number of ELISA-positive cows per herd and fecal-culture status (Table 2). An equal distribution of herds that were fecal-culture positive and negative was observed within the subcategory of herds that had at least one or two ELISA-positive cows per herd of 30 to 50 cows tested. A greater number of herds with three or more ELISA-positive cows were fecal-culture positive (79%) compared with herds with one (50%) or two (45.5%) ELISA-positive cows. Five herds (21%) that had three or more ELISA-positive cows were fecal-culture negative. Overall, 61% the study herds with at least one ELISA-positive cow were fecal-culture positive. A distribution of fecal-positive herds according to the number of ELISA-positive cows within the herd is depicted in Table 3. These data are further confirmation that herds with three or more ELISA-positive cows (of 30 to 50 tested) were more likely to be actively shedding *M. paratuberculosis* in their feces than those with one or two ELISA-positive cows.

The percentage of selected herds that were fecal-culture positive was also grouped according to number of

Table 3. Distribution of fecal-culture positive cows per herd compared with ELISA status within selected herds.

Number of culture-positive cows per herd	Number of ELISA-positive cows per herd ¹			
	1	2	≥3	Total
0	13 ²	6	5	24
1	6	1	3	10
2	4	0	2	6
3	2	1	3	6
4	0	0	3	3
5 or more	1	3	8	12

¹ χ^2 , 10 df = 6.022; $P < 0.111$.

²Number of herds.

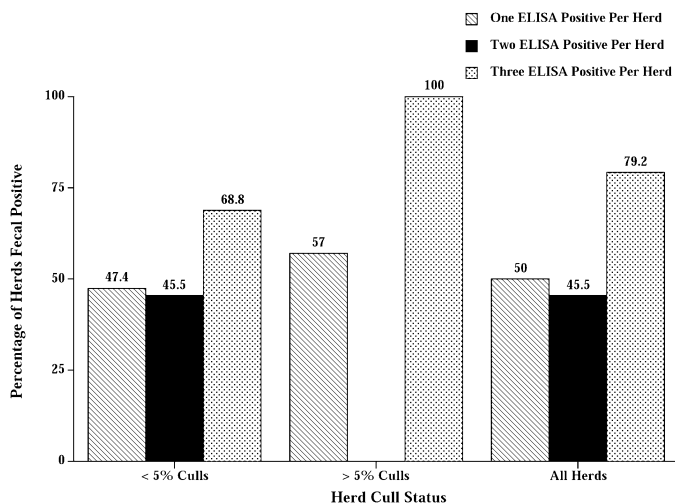


Figure 1. Percentage of fecal-culture-positive herds that had one, two, or three ELISA-positive cows within the herd, stratified according to herd history of culling because of Johne's disease in the previous year. N = 61 herds; χ^2 , 2 df = 3.7837, $P = 0.1508$.

ELISA-positive cows within the herd and herd cull status (Figure 1). Herds that reported <5% cull rate for Johne's disease in the previous year averaged 47, 45, and 69% of cows that were fecal-culture positive with one, two, or three or more cows that were ELISA-positive, respectively, within the herd. In contrast, herds with a higher cull rate for Johne's disease ($\geq 5\%$) averaged 57% fecal-culture positive with one ELISA-positive cow in the herd and 100% fecal-culture positive for herds with three or more ELISA-positive cows. Overall, 79% of selected herds with at least three ELISA-positive animals were fecal-culture positive.

Results from this study indicate that selection of animals within herds for fecal culture was evenly distributed between ELISA-positive and ELISA-negative cows. These data show that 55 of 158 (35%) ELISA-positive cows and 657 of 2144 (31%) ELISA-negative cows were selected for fecal culture. Selection of cows in the add-on study for fecal culture averaged 31% overall (712 of 2302). A total of 651 cows were tested by both fecal culture and ELISA, with 44 cows testing fecal-culture positive and 49 cows testing ELISA-positive (Table 4). A breakdown of cows that were fecal-culture positive showed that 11 of 44 (25%) of the cows were ELISA-positive and 33 of 44 (75%) were ELISA negative. A small percentage of animals that were fecal-culture negative tested ELISA-positive (6.3%). These data indicate an apparent prevalence rate of 6.8% for Johne's disease in the selected herds based on fecal-culture-positive cows. From previous NAHMS Dairy 96 ELISA testing, an apparent seroprevalence rate of 7.5% was obtained in these herds.

Table 4. Comparison of fecal-culture results with ELISA on a cow basis within 61 selected herds.

ELISA results	Fecal-culture results ¹		Total
	Positive	Negative	
Positive	11 ² (25%)	38 (6.3%)	49
Negative	33 (75%)	569 (93.7%)	602
Total	44	607	651

¹ χ^2 , 1 df = 20.699; $P < 0.01$.

²Number of cows.

We were unable to detect viable *M. paratuberculosis* in raw bulk tank milk samples after a culture period of 12 mo. However, PCR analysis of bulk tank milk demonstrated the presence of *M. paratuberculosis* DNA in 68% of samples as shown in Table 5. Approximately 77% (24 of 31) of herds with a subset of animals that were actively shedding *M. paratuberculosis* in their feces at the time of sample collection had PCR-positive milk samples. In contrast, 52.4% of herds that were fecal-culture negative were positive for the presence of *M. paratuberculosis* DNA in the milk. Overall, there was no interdependence between fecal shedding and the presence of *M. paratuberculosis* DNA in bulk tank milk samples.

DISCUSSION

The purpose of this study was manifold; the primary goal was to estimate the percentage of seropositive herds with detectable shedding of *M. paratuberculosis* in their feces and milk. The NAHMS Dairy Study conducted in 1996 provided the perfect opportunity to obtain useful information at the herd level with regard to these two tests. A total of 31,864 cows from 971 herds representing 20 different states were tested by ELISA for serum antibodies to *M. paratuberculosis* during the NAHMS study (Wells et al., 1998). These herds were not on a vaccination control program so interference

Table 5. Comparison of bulk tank milk polymerase chain reaction (PCR) results with fecal shedding within 52 selected herds.

Milk PCR	Fecal status of cows		Total
	Fecal positive ¹	Fecal negative ²	
Positive	24 ³ (77.4%)	11 (52.4%)	35
Negative	7 (22.6%)	10 (47.6%)	17
Total ⁴	31	21	52

¹At least one cow fecal-culture positive.

²All cows sampled fecal-culture negative.

³Number of herds.

⁴Milk samples were not received from all selected herds. χ^2 , 1 df = 3.5668; $P = 0.0589$.

with ELISA testing was not expected. Animals that are vaccinated often seroconvert and have measurable antibody levels to *M. Paratuberculosis*, which results in uncertainty as to whether the animal has seroconverted because of infection with the organism (Spangler et al., 1991).

The selection of cows from participating herds was random, and the percentage of cows selected for fecal culture in the add-on study was similar between ELISA-positive and ELISA-negative animals (35 vs. 31%). This confirms that our results were not biased by selecting only suspect cows from known positive herds and afforded us an opportunity to compare fecal culture with serologic responses at the herd level. In the present study, within the group of cows tested by both methods, only 25% of culture-positive cows were positive by ELISA. This is in contrast to previous reports that have suggested that the sensitivity of the ELISA test is approximately 45 to 50%, based on fecal-culture-positive animals (Collins and Sockett, 1993; Sweeney et al., 1995). Sweeney et al. (1995) further determined that sensitivity of the ELISA was dependent on the stage of infection of the animal being tested, ranging from 15 to 57% for subclinically infected cows and averaging 88% for clinically infected cattle shedding high numbers of organisms in their feces. In the present study, fecal-culture results spanned a wide range of colony counts for animals within herds (from one colony on one tube to too numerous to count on four tubes), indicating that a spectrum of disease states was represented. Inclusion of a high number of subclinically infected cows in the study would certainly adversely affect the sensitivity of ELISA because these animals have a reduced level of seroconversion. However, our results indicate that fecal-culture-positive herds were weighted heavily in favor of herds with three or more ELISA-positive cows (79%) so one would expect to detect a higher percentage of seropositive animals than we did.

Specificity of the commercial ELISA test for paratuberculosis has been reported to be as high as 99%, which suggests that in a herd of 100 cows only one cow will have a false-positive response in the test and will be inaccurately identified (Collins and Sockett, 1993). In the present study, a relative specificity of 94% in relation to fecal culture was obtained; 38 of 607 fecal-culture-negative cows were ELISA-positive. This finding was surprising and cannot be easily explained. Cross-reactivity of antigens within the ELISA test is one possibility. Animals infected with *M. paratuberculosis* do shed the microorganism intermittently in their feces and it is possible that some animals were misdiagnosed as fecal-culture negative because they were not shedding in their feces at the time samples were obtained.

However, intermittent shedding is more noteworthy in subclinically infected cows, which, in general, do not have measurable antibody titers to *M. paratuberculosis*. These results suggest a high percentage (6%) of false-positives in this study. Reduced specificities have been previously reported for ELISA tests by using capture antigens described as specific for *M. paratuberculosis* but typically have not been observed for the commercial ELISA kit used in this study (Abbas et al., 1983; Vannuffel et al., 1994). From the producer and veterinarian's perspective, estimates of positive and negative predictive values from this study were very interesting. Only 22% of ELISA-positive cows were fecal-culture positive [22% positive predictive value (PPV)]. Predictive value depends on the prevalence of infection within the herd, and this relatively low PPV should be expected in many dairy herds, as evidenced from the 61 dairy herds represented in this study.

Our inability to culture viable *M. paratuberculosis* from milk samples was not surprising, because the number of cows shedding the microorganism in their feces varied from 0 to 20% of selected cows within herds. It has been documented that cows with clinical disease or asymptomatic cows with heavy fecal shedding may shed less than 1 CFU/ml of milk (Sweeney et al., 1992). Compounding this low shedding rate into the milk, a significant dilution of milk occurs after collection from individual animals when combined with milk from the rest of the herd in the bulk tank. These factors make detection of the viable microorganism fairly difficult if not impossible. The sensitivity of the culture detection method used in our laboratory for raw milk samples is less than 10 CFU/ml of milk, yet this may have not been sensitive enough if the potential dilution of *M. paratuberculosis* in these samples is considered. However, we were able to successfully identify *M. paratuberculosis* in the bulk tank milk samples by PCR. Use of PCR allowed us to detect nonviable as well as viable microorganisms and would also be a more sensitive detection method because the *M. paratuberculosis* DNA is subjected to a double amplification during the nested PCR procedure. These results indicate that the dilution factor of *M. paratuberculosis* in bulk tank milk is fairly significant overall. This is an important point because it is currently unclear if *M. paratuberculosis* is a zoonotic agent that has similarities to Crohn's disease in human beings. Herds with a very high prevalence rate and with a large percentage of the cows shedding high numbers of *M. paratuberculosis* in their feces may yield a different result. Careful management of Johne's disease on farms would reduce the spread of infection and, subsequently, reduce any potential threat of animal-to-human transmission.

CONCLUSIONS

These results indicate that the sensitivity of the commercial ELISA test may be significantly lower than the previously reported 45 to 50% if one is basing sensitivity on positive fecal-culture results. Results from this study also yielded lower specificity estimates (94%) than previously acknowledged for the commercial ELISA test used here. Also, because of the relatively low prevalence of *M. paratuberculosis* infection, the PPV of the ELISA was very low in this study (22%). The reduced sensitivity or specificity estimates obtained in this study suggest that a high number of animals would be misdiagnosed if only the ELISA test is used to detect infection. In addition, contamination of bulk tank milk samples with *M. paratuberculosis* does occur in herds with animals that are actively shedding the microorganism, albeit in low numbers detectable by a PCR test.

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