Prevalence of antibodies against *Mycobacterium avium* subsp *paratuberculosis* among beef cow-calf herds

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Objective—To estimate the prevalence of *Mycobacterium avium* subsp *paratuberculosis* infection among cows on beef operations in the United States.

Design—Cross-sectional seroprevalence study.

Sample Population—A convenience sample of 380 herds in 21 states.

Procedures—Serum samples were obtained from 10,371 cows and tested for antibodies to *M avium* subsp *paratuberculosis* with a commercial ELISA. Producers were interviewed to collect data on herd management practices.

Results—30 (7.9%) herds had 1 or more animals for which results of the ELISA were positive; 40 (0.4%) of the individual cow samples yielded positive results. None of the herd management practices studied were found to be associated with whether any animals in the herd would be positive for antibodies to *M avium* subsp *paratuberculosis*.

Conclusions and Clinical Relevance—Results suggest that the prevalence of antibodies to *M avium* subsp *paratuberculosis* among beef cows in the United States is low. Herds with seropositive animals were widely distributed geographically. (*J Am Vet Med Assoc* 2001;219:497–501)

Mycobacteriosis (Johne's disease) is a chronic progressive disease of ruminants worldwide caused by *Mycobacterium avium* subsp *paratuberculosis*. The effects of this disease on beef cattle production are a result of premature culling of affected animals, decreased milk production leading to reduced weaning weights of calves, reduced body weight of culled animals, and loss of potential markets for breeding animals domestically and internationally. Although these losses have not been well quantified for beef herds, extrapolation from experiences with dairy herds indicate that in some instances these impacts could be substantial.[1]

Previous estimates of the prevalence of mycobacteriosis have ranged widely, depending on the source of the samples examined and the method used to detect antibodies to *M avium* subsp *paratuberculosis* or the organism itself. Use of nonvalidated tests, insensitive methods, and selected groups of animals all contribute to the variability in reported estimates of prevalence. The estimated national prevalence of *M avium* subsp *paratuberculosis* infection in beef cows, determined by means of bacterial culture of ileocecal lymph nodes collected from beef cows at the time of slaughter during 1983 and 1984, was 0.8%.[2] A seroprevalence study[3] of beef cows in Oklahoma, using samples collected for routine brucellosis surveillance, reported that 1% of cows had antibodies to *M avium* subsp *paratuberculosis*. An unpublished study[a] of beef cows in Idaho in which blood samples were collected at the time of slaughter for serologic testing, along with fecal samples for bacterial culture and sections of intestine for histologic evaluation, found that 1.0% of the beef cows were seropositive, but only 0.4% had histologic evidence of infection; results of 1 or both tests were positive for 1.1% of the beef cows studied. A seroprevalence study[4] in Florida found that 8.6% of beef cattle had antibodies to *M avium* subsp *paratuberculosis*. To our knowledge, however, there have been no estimates of the herd-level prevalence of mycobacteriosis in beef cow-calf operations, and aside from the study by Merkal et al,[2] there have been no studies of the prevalence of *M avium* subsp *paratuberculosis* infection in beef cows from a wide geographic region.

In 1997, the USDA:APHIS **National Animal Health Monitoring System (NAHMS)** performed a comprehensive study[5] of the health and management of the cow-calf segment of the beef industry. The present report represents a portion of that study; the purpose of this portion of the NAHMS study was to estimate the prevalence of *M avium* subsp *paratuberculosis* infection on beef operations in the United States.

Materials and Methods

Source of samples—The methods for the NAHMS study of the beef cow-calf industry have been described.[5] Briefly, a stratified random sample of cow-calf operations believed to have beef cows in 23 states[b] was selected. Producers were contacted by trained enumerators from the National Agricultural Statistics Service who collected general information on animal production practices. Operations that participated in the first phase of the study and that had at least 5 beef cows were eligible to participate in the second phase of the study. During the second phase of the study, a federal or state veterinarian or animal health technician contacted the producer to collect more information on production practices, health management, and health status of cattle on the

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operation. In addition, in 21[c] of the 23 states, producers were asked to allow investigators to collect blood samples from cows for detection of antibodies to *M avium* subsp *paratuberculosis*. The number of samples collected from each herd depended on the number of beef cows in the herd; the sampling scheme was derived with the goal that we would be 90% certain of detecting at least 1 seropositive animal in a herd if the true prevalence for that herd was 10% and sensitivity and specificity of the assay to detect antibodies were 50 and 99%, respectively. Samples were collected from all cows in the herd if the herd consisted of ≤ 25 cows, 25 cows if the herd consisted of 26 to 49 cows, 30 cows if the herd consisted of 50 to 99 cows, 35 cows if the herd consisted of 100 to 249 cows, and 40 cows if the herd consisted of ≥ 250 cows. Blood samples were submitted to a central laboratory, where serum was harvested and kept frozen at –20 C until tested.

Sample testing—All samples were tested with a commercial ELISA[d] performed according to the manufacturer's instructions, except that samples were tested in single rather than duplicate wells. Prior to testing, serum samples were diluted 1:20 with the diluent provided by the kit's manufacturer. Wash steps were completed with an automated plate washer.[e] Plates were read with an automated reader[e] at a wavelength of 650 nm. For each sample, a **test sample-to-positive control sample (S:P)** ratio was calculated as the test sample **optical density (OD)** minus the mean OD for the negative control samples divided by the mean OD of the positive control samples minus the mean OD of the negative control samples. Results were considered positive if the S:P ratio was ≥ 0.25. Plates for which the mean OD for the negative control samples was > 0.12 were considered invalid, as were plates for which the difference between the mean OD for the negative control samples and the mean OD for the positive control samples was < 0.15. All tests were performed during a 13-day period, using test kits from a single manufacturing lot (121GR).

Data analysis—States were grouped into 5 regions,[f] and percentages of seropositive herds in the 5 regions were compared with a χ^2 test.[6] Values for categorical data were compared between seropositive and seronegative herds with χ^2 or Fisher exact tests.[6] Because of the small number of seropositive herds, values for continuous data were compared between seropositive and seronegative herds with the Wilcoxon test.[7]

To assess the potential for nonresponse bias in the study, herds from which blood samples were collected were compared with the remaining herds from the 21 states in which those herds were located. Values for categorical and continuous data were compared between herds from which blood samples were collected and herds from which blood samples were not collected with χ^2 tests and *t*-tests,[8] respectively. All statistical analyses were carried out with commercially available software.[g] For all analyses, a value of $P \leq 0.05$ was considered significant.

Results

A total of 1,190 operations were eligible to participate in the second phase of the NAHMS study, which included collection of questionnaire data and a request to collect blood samples. Overall, 10,371 blood samples were collected from cows on 380 operations. Results of the ELISA were positive for 40 samples (0.4%), representing cows on 30 (7.9%) operations. For 22 of the 30 seropositive operations, results were positive for only a single test sample. For 6 operations, results were positive for 2 samples each, and for 2 operations, results were positive for 3 samples each. Most samples for which results were positive had S:P ratios ≥ 0.30 (34/40 [85%]; **Table 1**). Most samples for which results were negative had S:P ratios < 0.001 (9,325/10,331 [90.3%]).

Table 1—Results of testing of serum samples from 10,371 beef cows for antibodies to *Mycobacterium avium* subsp *paratuberculosis*

S:P ratio*	No. of tests	Percentage
< 0.001	9,325	89.91
0.001 to 0.09	897	8.65
0.1 to 0.19	91	0.88
0.2 to 0.249	18	0.17
0.25 to 0.29	6	0.06
0.3 to 0.39	7	0.07
0.4 to 0.59	6	0.06
≥ 0.6	21	0.20

*The S:P ratio represents the test sample-to-positive control sample ratio; samples for which the S:P ratio was ≥ 0.25 were considered to be positive for antibodies to *M avium* subsp *paratuberculosis*.

Table 2—Comparison of management and production factors for 30 beef herds with 1 or more cows with antibodies to *M avium* subsp *paratuberculosis* (seropositive herds) and 350 beef herds not found to have any cows with antibodies to *M avium* subsp *paratuberculosis* (seronegative herds)

Factor	Seropositive herds	Seronegative herds	*P* value
Mean No. of cows	109	139	0.4
Registration status*			0.85
Registered	1/30 (3)	19/347 (5.5)	
Commercial	22/30 (73)	241/347 (69.5)	
Both	7/30 (23)	87/347 (25)	
Purebred bull purchased within past 5 years*			0.4
Yes	28/30 (93)	298/348 (85.6)	
No	2/30 (7)	50/348 (14.4)	
Embryo transfer*			1.0
Yes	1/30 (3)	12/348 (3.5)	
No	29/30 (97)	336/348 (96.5)	
Holstein recipients for embryo transfer*			0.31
Yes	1/1 (100)	3/12 (25)	
No	0/1 (0)	9/12 (75)	
Embryo transfer 3 to 5 years ago*			0.6
Yes	1/30 (3)	10/346 (2.9)	
No	29/30 (97)	336/346 (97.1)	
Holstein recipients for embryo transfer 3 to 5 years ago*			1.0
Yes	1/1 (100)	5/10 (50)	
No	0/1 (0)	5/10 (50)	
Separate cow-calf pairs from pregnant cows*			0.7
Yes	10/30 (33)	132/348 (37.9)	
No	20/30 (67)	216/348 (62.1)	
Mean weaning weight (kg)			
Bulls and steers	245	241	0.89
Heifers	221	222	0.69
Familiarity with Johne's disease*			0.04
Never heard of it	17/30 (57)	192/348 (55.2)	
Recognized name	6/30 (20)	118/348 (33.9)	
Knew basics	6/30 (20)	21/348 (6.0)	
Fairly knowledgeable	1/30 (3)	17/348 (4.9)	
Mean culling percentage	12	10.6	0.22
Region*			0.03
West	3/30 (10)	116/350 (33.1)	
North central	9/30 (30)	71/350 (20.3)	
South central	1/30(3)	34/350 (9.7)	
Central	8/30 (27)	51/350 (14.6)	
Southeast	9/30 (30)	78/350 (22.3)	

*Data are given as No. of herds with that factor/No. of herds for which information was available (%).

Table 3—Comparison of management and production factors for beef herds from which blood samples were obtained for testing for antibodies to *M avium* subsp *paratuberculosis* (tested herds) and beef herds from which blood samples were not obtained (untested herds)

Factor	Tested herds	Untested herds	*P* value
Mean No. of cows	136.87	109.5	0.04
Registration status*			0.52
Registered	20/377 (5.3)	29/722 (4.0)	
Commercial	263/377 (69.8)	522/722 (72.3)	
Both	94/377 (24.9)	171/722 (23.7)	
Purebred bull purchased within past 5 years*			0.77
Yes	326/378 (86.2)	618/722 (85.6)	
No	52/378 (13.8)	104/722 (14.4)	
Embryo transfer*			0.83
Yes	13/378 (3.4)	23/721 (3.2)	
No	365/378 (96.6)	698/721 (96.8)	
Holstein recipients for embryo transfer*			1.0
Yes	4/13 (31)	7/23 (30)	
No	9/13 (69)	16/23 (70)	
Embryo transfer 3 to 5 years ago*			0.42
Yes	11/376 (2.9)	28/721 (3.9)	
No	365/376 (97.1)	693/721 (96.1)	
Holstein recipients for embryo transfer 3 to 5 years ago*			0.49
Yes	6/11 (55)	11/27 (41)	
No	5/11 (45)	16/27 (59)	
Separate cow-calf pairs from pregnant cows*			0.04
Yes	142/378 (37.6)	226/722 (31.3)	
No	236/378 (62.4)	496/722 (68.7)	
Mean weaning weight (kg)			
Bulls and steers	241	237	0.19
Heifers	222	218	0.19
Familiarity with Johne's disease*			0.04
Never heard of it	209/378 (55.3)	459/722 (63.6)	
Recognized name	124/378 (32.8)	190/722 (26.3)	
Knew basics	27/378 (7.1)	50/722 (6.9)	
Fairly knowledgeable	18/378 (4.8)	23/722 (3.2)	
Mean culling percentage	10.7	11.8	0.57
Region*			< 0.01
West	118/378 (31.2)	143/722 (19.8)	
North central	79/378 (20.9)	136/722 (18.8)	
South central	35/378 (9.3)	146/722 (20.2)	
Central	59/378 (15.6)	137/722 (19.0)	
Southeast	87/378 (23.0)	160/722 (22.2)	

*Data are given as No. of herds with that factor/No. of herds for which information was available (%).

The percentage of seropositive herds was highest for the north central region and lowest for the south central region (**Table 2**). The regional distribution of seropositive herds was significantly ($P = 0.03$) different from the regional distribution of seronegative herds. Approximately equal percentages of herds with seropositive animals as herds without seropositive animals reported having never heard of Johne's disease previous to this study; however, producers of herds with seropositive animals had higher levels of knowledge about the disease than did producers of herds without seropositive animals. Herds with 1 or more seropositive animals were not significantly different from herds without any seropositive animals in regard to median herd size, median estimated mean weaning weights for steers and bulls or for heifers, registration status of the cow herd (commercial, registered, or mixed), whether the producer had purchased any purebred bulls in the past 5 years, use of embryo transfer now or in the past with or without Holstein recipients, and whether the herd separated cow-calf pairs from pregnant cows after calving.

The distribution of herds from which blood samples were collected was not significantly different from the distribution of herds that declined to allow investigators to collect blood samples in regard to registration status of the herd (commercial, registered, or mixed), purchase of purebred bulls in the past 5 years, use of embryo transfer now or in the past with or without Holstein recipients, median estimated mean weaning weights for steers and bulls or for heifers, and median estimated culling percentage (**Table 3**). Herds from which blood samples were collected were significantly larger, more likely to separate cow-calf pairs from pregnant cows shortly after birth, and more likely to have known something about Johne's disease than those from which blood samples were not collected. Percentages of herds from which blood samples were collected varied significantly among regions.

Discussion

Relative to other studies of beef and dairy cattle, few animals and operations in the present study were seropositive for antibodies to *M avium* subsp *paratuberculosis*. However, comparisons to other studies should be made with caution because of differences in tests used and analysis methods. For example, in a previous national study[9] of the prevalence of *M avium* subsp *paratuberculosis* antibodies in dairy cattle, a herd was classified as seropositive only if ≥ 2 animals were seropositive or if 1 animal was seropositive and ≥ 5% of the animals culled in the previous year had signs consistent with mycobacteriosis.

The low apparent seroprevalence in the present study may reflect an overall low prevalence of infection among beef cattle in the states where samples were collected. Beef cattle are generally managed in a more extensive manner than are dairy cattle, which may provide fewer opportunities for transmission of the organism. However, several aspects of the study design may have affected the observed seroprevalence. Most importantly, if the true within-herd prevalence was < 10% and the true test sensitivity was < 50%, then the probability of detecting 1 or more seropositive animals in a seropositive herd would be < 90% with the sampling scheme we used. Given the extensive manner in which beef cattle are typically managed, it is feasible that the within-herd prevalence is < 10%, and recent estimates of the true sensitivity of this test are < 50%.[10] Thus, the herd prevalence of 7.9% in the present study may be an underestimate of the true overall herd prevalence.

For the most part, there was good separation of the S:P ratios for blood samples with positive and negative results, giving us confidence that most, if not all, of the positive results were true-positive results and that most of the negative results were true-negative results. During the course of infection, one may expect some infected animals to have S:P ratios just below the cutoff for a positive result. In the present study, however, there were few samples with S:P ratios in this range, indicating that there likely was not a large population

of animals that could be expected to become seropositive in the near future.

The percentage of samples for which results were positive was less than what may have been expected from testing this large number of samples with a test with a reported specificity of 99%.[11,12] If the specificity were truly 99%, we would have expected approximately 100 (95% confidence interval, 84 to 124) false-positive test results. The fact that results were positive for only 40 samples and that for most of these 40 samples the S:P ratio was high suggests that the specificity of this test in beef cattle could be > 99%. Further studies should be conducted to determine whether the test performs differently in beef cattle than it does in dairy cattle, on which virtually all studies of test validation have been performed.[11-13] In addition, further tests should evaluate the performance of other manufacturing lots of the kit.

Participation in the NAHMS study was voluntary, and many producers (808/1,189 [68%]) elected to not have blood samples collected. Given this nonresponse rate, there was an opportunity for bias in the sample. To evaluate the potential for this type of bias in the sample, herds from which blood samples were collected were compared with those from which blood samples were not collected. One important difference we discovered was that herds from which blood samples were collected were larger than herds from which blood samples were not collected. We believe that this represents a bias toward herds that typically handle their cattle for various reasons, making them more accessible for collection of blood samples. Similarly, response rate was related to geographic location of the herd. Again, this was likely related to routine handling of animals for other reasons, which affected the availability of animals for collection of blood samples.

Producers who had blood samples collected from their animals tended to have more knowledge of Johne's disease than did producers who did not have blood samples collected. Apparently, therefore, knowledge of Johne's disease was not a deterrent to these producers to participating in the sample collection part of the study.

We did not detect any other important differences between herds from which blood samples were collected and herds from which blood samples were not collected in regard to management and production factors, making it likely that the herds included in the study were similar to the herds that were not included. However, the distribution of herds included in the study may be different from the overall population with respect to some factors. Because of this potential bias and the lower confidence in detecting herds with a low prevalence of infection, it is best, therefore, to treat the estimates from this study as having wide confidence intervals and providing only a very general indication of the true state of the population.

Some have hypothesized that *M avium* subsp *paratuberculosis* infection may be more common in registered herds, compared with commercial herds, because of the increased movement of cattle as well as the use of procedures such as embryo transfer with Holstein recipients and the use of nurse cows. In the present study, only 4% of the operations were registered operations, which prevented us from evaluating this hypothesis with any statistical power.

Test performance could certainly affect the number of samples for which results were positive and, thus, the estimates of the prevalence of this disease. To address this concern, we have evaluated the sensitivity and specificity of the currently available test.[13] Sensitivity of this test varies with stage of infection, as has been demonstrated for another ELISA for detection of antibodies to *M avium* subsp *paratuberculosis*.[12] The current test has approximately equivalent characteristics to a previously available ELISA,[13] and there is no information to indicate that test performance adversely affected results.

To the extent that operations from which samples were obtained in the present study represent a geographically diverse group of beef operations, the prevalence of *M avium* subsp *paratuberculosis* infection in beef cows appears to be low. In addition, although the percentage of herds with cows infected with *M avium* subsp *paratuberculosis* varies with geographic region, percentages appear to generally be low. Further work to evaluate the prevalence of *M avium* subsp *paratuberculosis* infection in subgroups of cattle (eg, particular types of cattle, geographic areas, or types of management systems) would be helpful to target industry efforts to deal with infections with this organism.

[a]England JJ, Caine Veterinary Teaching Hospital, University of Idaho, Caldwell, Idaho: Personal communication, 1999.
[b]Alabama, Arkansas, California, Colorado, Florida, Georgia, Illinois, Iowa, Kansas, Kentucky, Montana, Missouri, Mississippi, Nebraska, New Mexico, North Dakota, Oklahoma, Oregon, South Dakota, Texas, Tennessee, Virginia, and Wyoming.
[c]Florida and Oklahoma were not included.
[d]IDEXX Laboratories, Westbrook, Me.
[e]Tecan US Inc, Research Triangle Park, NC.
[f]West = California, Colorado, Montana, New Mexico, Oregon, and Wyoming; north central = Kansas, Nebraska, North Dakota, and South Dakota; central = Illinois, Iowa, and Missouri; south central = Texas; southeast = Alabama, Arkansas, Georgia, Kentucky, Mississippi, Tennessee, and Virginia.
[g]SAS for personal computers, SAS Institute Inc, Cary, NC.

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