Prevalence of and risk factors for paratuberculosis in purebred beef cattle

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Objective—To estimate the prevalence of paratuberculosis in purebred beef cattle in Texas and identify risk factors for seropositivity.

Design—Epidemiologic survey.

Animals—4,579 purebred cattle from 115 beef ranches in Texas.

Procedure—Blood was collected, and serum was analyzed for antibodies with a commercial ELISA. Fecal samples were collected and frozen at –80°C until results of the ELISA were obtained, and feces from seropositive cattle were submitted for mycobacterial culture. Herd owners completed a survey form on management factors.

Results—Results of the ELISA were positive for 137 of the 4,579 (3.0%) cattle, and 50 of the 115 (43.8%) herds had at least 1 seropositive animal. Results of mycobacterial culture were positive for 10 of the 137 (7.3%) seropositive cattle, and 9 of the 50 (18%) seropositive herds had at least 1 animal for which results of mycobacterial culture were positive. Risk factors for seropositivity included water source, use of dairy-type nurse cows, previous clinical signs of paratuberculosis, species of cattle (*Bos taurus* vs *Bos indicus*), and location.

Conclusions and Clinical Relevance—Results suggested that seroprevalence of paratuberculosis among purebred beef cattle in Texas may be greater than seroprevalence among beef cattle in the United States as a whole; however, this difference could be attributable to breed or regional differences in infection rates or interference by cross-reacting organisms. Veterinarians should be aware of risk factors for paratuberculosis as well as the possibility that unexpected serologic results may be found in some herds. (*J Am Vet Med Assoc* 2005;226:773–778)

There are few studies of the prevalence of paratuberculosis caused by ***Mycobacterium avium* subsp *paratuberculosis* (MAP)** in beef cattle, but it is generally considered to be a disease of importance only in dairy cattle. Two studies[1,2] conducted in the 1980s reported that 4.4% of beef cattle in Louisiana and 8.8% of beef cattle in Florida were seropositive, but ELISAs used in those studies were not as specific as assays available today. In a separate study,[3] MAP was isolated from the ileocecal lymph nodes of 0.8% of beef cattle evaluated at a slaughterhouse in 1983 and 1984. The most recently published national studies[4,5] of paratuberculosis in the United States reported that seroprevalences of paratuberculosis among dairy cattle in 1996 and beef cattle in 1997 were 2.5% and 0.4%, respectively, with at least 22% of dairy herds and 7.8% of beef herds having at least 1 seropositive animal. Because of the perceived lower prevalence of paratuberculosis in beef cattle and the lower level of awareness about paratuberculosis among beef producers, the disease has been given a low priority by beef cattle producers and veterinarians for many years.

In the late 1990s, the National Johne's Working Group and the Johne's Committee of the United States Animal Health Association began to formulate plans for a comprehensive paratuberculosis control program. When the members of the Texas Johne's Working Group began to meet and share opinions and experiences, it became apparent that the national estimate of the prevalence of paratuberculosis was lower than that perceived by veterinarians and cattle producers on the committee. In response to the urging of the beef cattle industry in Texas, which represents > 30% of all beef cattle in the United States, we decided to investigate the prevalence of paratuberculosis in purebred beef cattle. We focused on this subset of the beef cattle population because we suspected that the prevalence would be highest in these cattle. We also believed that the economic impact and potential for dissemination would be highest in this subpopulation. Specifically, the purposes of the study reported here were to estimate the seroprevalence of paratuberculosis in purebred beef cattle in Texas, determine herd infection rates on the basis of results of mycobacterial culture of fecal samples, identify specific ranch management practices

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Supported by Texas A&M University College of Veterinary Medicine Signature Program Funds, the Houston Livestock Show and Rodeo, the San Antonio Stock Show and Rodeo, the Southwestern Exposition Livestock Show and Rodeo, Hi-Pro Distributors, Fort Dodge Laboratories, and Idexx Laboratories Incorporated.

Presented in part at the 7th International Colloquium for Paratuberculosis, Bilbao, Spain, June 2002 and the 21st Annual American College of Veterinary Internal Medicine Forum, Charlotte, NC, June 2003.

The authors thank Drs. Arturo Angulo and Loyd Sneed and Elizabeth Stewart, Terry Fyock, Phillip Petersen, Kim Eisenberg, and Tiffany Cadenhead for technical assistance; Dr. David Smith for consultation; and Dr. Richard Forguson and Richard Traylor for assistance in acquiring funding.

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associated with the risk of paratuberculosis in beef cattle, and estimate geographic risk patterns for paratuberculosis in Texas.

Materials and Methods

Recruitment and selection of beef herds—The study was conducted between September 1, 2000, and March 1, 2001. Membership lists were obtained from 14 breed associations in Texas, and > 7,000 letters soliciting participation in the study were mailed to purebred beef cattle producers. Individual producers were eligible for inclusion in the study if they had ≥ 25 purebred cattle in Texas, were willing to complete the study survey form, and were willing to have their cattle tested during the study period. The authors had no prior estimates as to the relative roles of spatial and random herd effects and, thus, did not develop the sample strategy on the basis of standard calculations. All fully compliant herds were included in the study, reflecting this absence of useful prior information. The study protocol was approved by the Clinical Research Review Committee of Texas A&M University.

Sample collection and analysis—The attending veterinarians for producers enrolled in the study were paid to collect study samples. For each herd, blood and fecal samples were collected from all cattle ≥ 2 years old, up to 50 cattle/herd. For herds that contained > 50 eligible cattle, the owner was asked to select the 50 cattle from which samples were collected. The owner was instructed to avoid bias in selecting cattle, but truly random sampling was not attempted. The number of samples collected per herd was chosen to give each herd owner a valuable appraisal of the herd's serostatus. Previous calculations have suggested that even with very large herds, collecting samples from 30 animals/herd will result in ≥ 1 positive test result for at least 95% of herds in which the seroprevalence of paratuberculosis is at least 10%.

Blood was collected into plain evacuated tubes, and fecal samples were collected into sterile plastic bags; all samples were shipped on ice to the Texas Veterinary Medical Diagnostic Laboratory by express courier. All samples arrived within 5 days after collection. Serum was separated from the blood samples and analyzed immediately, if possible, or frozen at –80°C until analyzed. Serum was tested with a commercially available ELISA kit[a] according to the manufacturer's directions; results were considered positive if the test sample-to-positive control sample (S:P) ratio was ≥ 0.25.

Fecal samples were frozen at –80°C until results of the ELISA were obtained. Feces from seropositive cattle were thawed, and a portion was submitted to the bacteriology laboratory at the Texas Veterinary Medical Diagnostic Laboratory. The remainder of each fecal sample was immediately refrozen at –80°C. A double centrifugation method was used to isolate MAP. Herrold's egg yolk medium with and without mycobactin J[b] was inoculated and incubated for 15 weeks at 37°C. Mycobactin J-dependent, acid-fast organisms were confirmed to be MAP by means of a polymerase chain reaction assay. After mycobacterial culture of all fecal samples was completed at the Texas Veterinary Medical Diagnostic Laboratory, the remaining portions of the fecal samples were sent to the University of Pennsylvania, where a centrifugation technique with incubation on Herrold's egg yolk medium for 16 weeks was used to isolate MAP. Any colonies that appeared were subcultured without mycobactin J. Mycobactin J-dependent colonies were acid-fast stained for confirmation of their identity.

Collection and analysis of survey data—Owners of herds participating in the study were required to complete a survey[c] eliciting information on management practices. Survey questions were related to breed, herd size, source of animals, animal traffic, grazing and calving areas, breeding systems, feeding and watering practices, and previous history of paratuberculosis or clinical signs consistent with paratuberculosis.

Data analysis—Prior to analysis, survey responses were coded. For species classification, 3 categories were identified: *Bos indicus*, *Bos taurus*, and interspecies. The interspecies category included Beefmaster, Brangus, Santa Gertrudis, Braford, and Simbrah. Use of embryo transfer recipients was categorized as did not use embryo transfer, used only beef-type cows as embryo transfer recipients, and used at least 1 dairy-type cow as an embryo transfer recipient. Continuous variables, including cattle density (ie, cows or cow-calf pairs/acre) during the calving season and during the non-calving season and the number of cattle sold at purebred cattle sales, were dichotomized at their median value (ie, higher or lower than median value). Use of seasonal calving patterns was categorized as did not use seasonal calving (ie, used year-round breeding), spring calving, fall calving, and both spring and fall calving. Each ranch was georeferenced by use of a US Postal Service database that provided latitude and longitude from a physical address.

Risk modeling—The outcome modeled was individual animal risk for seropositivity. Modeling of risk factors was performed in 3 stages. In stage 1, all factors were analyzed individually for association with seropositivity by means of logistic regression. Overdispersion of herd effects was accounted for by inflating the variance estimate by a factor calculated as deviance divided by the deviance degrees of freedom.

In stage 2, some risk factors were recoded for parsimony. Use of seasonal calving was reduced from 4 categories to 2 (spring calving vs all other patterns of calving). Species was reduced to a linear code with *Bos taurus*-based herds coded 0, *Bos indicus*-based herds coded 1, and interspecies herds coded 0.5. All factors proposed as possibly causal and significant at a *P* value cutoff of 0.1 were entered into a multivariate model, and a backward stepwise elimination procedure was used until all factors were significant at a *P* value cutoff of 0.1. Specifically, an owner's observation of clinical signs of paratuberculosis was not considered possibly causal and was not included in stage 2 modeling. This ensured that this factor would not be identified as a possible causal factor and, therefore, would not obscure the effects of a factor that did cause seropositivity. As in the univariate model, the response (seropositivity of an individual animal) was assumed to fit an overdispersed binomial distribution, and logistic regression was performed with the variance of the logit of the relative risk adjusted by a factor equal to the deviance divided by the deviance degrees of freedom.

In stage 3, factors significant at a *P* value cutoff of 0.1 in the second stage were modeled as covariates and these covariates were adjusted for spatial risk. The adjusted **odds ratios (ORs)** for covariates and the spatial risk were estimated by use of Bayesian generalized linear Kriging[6] as expanded to include extrabinomial variation at the cluster level and cluster-level covariates.[7] Briefly, the model incorporated a Bayesian method of inference with vague priors and a Markov-chain Monte Carlo implementation. The Monte Carlo implementation was performed with standard software.[d] Priors included a flat improper prior for the intercept and vague gamma priors for variance components including the range, nugget (spatially random herd effect), and spatial effects (spatially dependent herd effect). The initial 500 iterations were discarded to allow for convergence. Convergence was determined by starting 2 chains with disparate initial values to observe for convergence. Derivative-

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free adaption rejection sampling was performed; 10,000 iterations were retained for estimation of ORs, and 1,000 iterations were retained for estimation of spatial risk. The variance function model chosen was exponential with the model covariance between $farm_i$ and $farm_j$ defined as a function of the distance between the 2 farms (d_{ij}) and the rate of decline of the covariance (ϕ; range parameter) defined as follows: $f(d_{ij}, \phi) = \exp(-[\phi d_{ij}])$.

Mapping—Bayesian spatial prediction was performed for a grid of points encompassing the entire state with each point representing the centroid of a 10 × 10-km area. The predicted surface was plotted with commercially available mapping software.[e]

Results

A total of 648 purebred beef cattle producers responded to the letter soliciting participation in the study. Of these, 313 met the inclusion criteria and were invited to participate. Ultimately, 115 ranches were included in the study. Of these, 114 completed the survey form, 111 were successfully georeferenced, and 115 submitted blood and fecal samples.

Blood samples were collected from 4,579 cattle. Results of the ELISA were positive for 137 of the 4,579 (3.0%) cattle, and 50 of the 115 (43.8%) herds had at least 1 seropositive animal. Results of mycobacterial culture of feces were positive for 10 of the 137 (7.3%) seropositive cattle, and 9 of the 50 (18%) seropositive herds had at least 1 animal for which results of mycobacterial culture were positive. Although only fecal samples from seropositive cattle were cultured, the 10 cattle for which culture results were positive represented 0.2% of the 4,579 cattle in the study and the 9 herds with at least 1 animal with positive culture results represented 7.8% of the 115 herds in the study.

Sixty-three of the 469 (13.4%) *Bos indicus* cattle, 52 of the 1,594 (3.3%) interspecies cattle, and 22 of the 2,416 (0.9%) *Bos taurus* cattle were seropositive. All 11 (100%) of the *Bos indicus*-based herds, 24 of the 39 (61.5%) interspecies-based herds, and 15 of the 65 (23%) *Bos taurus*-based herds had at least 1 seropositive animal. For most of the herds with at least 1 seropositive animal, seroprevalence ranged from 2% to 4%. However, in 9 herds seroprevalence was ≥ 12%. Of these, 6 were *Bos indicus*-based, 2 were interspecies-based, and 1 was *Bos taurus*-based.

Mycobacterium avium subsp *paratuberculosis* was isolated from 10 fecal samples at the first laboratory and from 4 fecal samples at the second laboratory. For all 4 samples from which MAP was isolated at the second laboratory, MAP was also isolated at the first laboratory. These 10 cattle for which results of mycobacterial culture were positive included 3 of the 63 (4.8%) seropositive *Bos indicus* cattle, 5 of the 52 (9.6%) seropositive interspecies cattle, and 2 of the 22 (9%) seropositive *Bos taurus* cattle.

Stage 1 of the risk modeling analysis indicated that seropositivity was significantly ($P < 0.1$) related to use of a dairy-type nurse cow, seasonal calving pattern, water source, species of cattle, and previous recognition of clinical signs of paratuberculosis **(Table 1)**. Seasonal calving pattern was a significant risk factor regardless of whether it was included in the model with 4 categories (no seasonal calving vs spring calving vs fall calving vs spring and fall calving) or with only 2 categories (spring calving vs any other calving pattern). Similarly, cattle species was a significant risk factor regardless of whether it was included in the model with 3 categories or as a linear code. However, including cattle species as a linear code improved risk modeling.

Stage 2 modeling showed that when controlling for the effects of cattle species and water source (streams and rivers vs any other source), use of a dairy-type nurse cow and seasonal calving pattern were not significantly ($P > 0.1$) associated with risk of seropositivity. However, cattle species (linear code; OR, 16.87; 95% **confidence interval [CI]**, 9.07 to 31.40) and water source (streams and rivers vs any other source; OR, 1.65; 95% CI, 0.96 to 2.85) were still identified as risk factors for seropositivity.

Even when controlling for spatial risks in stage 3 of modeling, cattle species (linear code; OR, 21.23; 95% CI, 8.22 to 49.41) and water source (streams and rivers vs any other source; OR, 2.12; 95% CI, 1.01 to 4.03) were still identified as risk factors for seropositivity.

Mapping of spatial risk identified areas of high and low risk **(Figure 1)**. The region of greatest risk for seropositive herds was central-eastern and southeastern Texas.

Table 1—Results of univariate analysis of the individual animal odds of seropositivity for paratuberculosis among purebred beef cattle in Texas.

Factor	Odds ratio	95% Confidence interval
Use of a dairy-type nurse cow	2.085	0.926–4.306
Running stream or river vs other water source	2.167	1.008–5.315
Use of seasonal calving (4 categories)		
Spring and fall calving vs no seasonal calving	0.976	0.402–2.763
Fall calving vs no seasonal calving	0.413	0.026–2.383
Spring calving vs no seasonal calving	2.288	0.854–6.846
Use of seasonal calving (spring calving vs all other patterns)	2.469	1.160–4.947
Previous clinical signs (yes vs no)	2.768	1.472–5.381
Species (3 categories)		
Bos indicus vs *Bos taurus*	17.418	8.720–37.465
Interspecies vs *Bos taurus*	3.567	1.785–7.655
Species (linear code; *Bos indicus* vs *Bos taurus*)	18.558	9.151–38.778

Data represent results of an ELISA performed on 4,579 cattle in 115 herds. Modeling allowed for overdispersion of herd effects.

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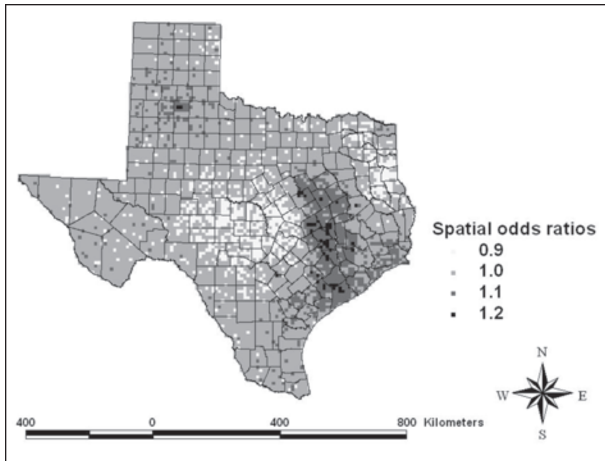

Figure 1—Map indicating spatial risk of seropositivity for paratuberculosis among purebred beef cattle herds in Texas. The odds ratio is plotted relative to the overall expected odds for seropositivity with odds ratios > 1 reflecting higher than expected risk and odds ratios < 1 reflecting lower than expected risk.

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Discussion

The seroprevalence of paratuberculosis among beef cattle in the present study was higher than that reported in a previous study[5] of beef cattle. The difference in results between the 2 studies may be attributable to the subsets of beef cattle that were tested or to the geographic region where cattle were located. The present study was limited to herds of purebred beef cattle, whereas in the previous study,[5] only 4% of participating herds had registered cattle and 25% had registered and commercial cattle. Several conditions may make purebred cattle more likely to contract infectious diseases, including mixing of cattle. For example, the use of dairy cattle as embryo transfer recipients or nurse cows and higher cattle density at calving and during grazing could increase exposure to pathogens. Alternatively, the higher prevalence in the current study may be attributable to geographic differences in risk. Results of the present study suggest that there is an area with a high risk for seropositivity in central Texas.

Species distribution may also have contributed to the difference between results of the present and a previous study.[5] A large number of the cattle in the present study were *Bos indicus* or interspecies cattle, and seroprevalence in these breeds was higher than seroprevalence in *Bos taurus* cattle. In the present study, 10% of the cattle were *Bos indicus* cattle and 36% were interspecies cattle, which reasonably represents the breed distribution in Texas and the Gulf Coast region, but does not represent the national breed distribution. A total of 22 of the 2,416 (0.9%) *Bos taurus* cattle in the present study and 15 of the 65 (23%) *Bos taurus*-based herds were seropositive, whereas 0.4% of cattle and 8% of herds in the previous study[5] were seropositive. The difference in percentages of herds with at least 1 seropositive animal could also be related in part to the greater number of cattle we tested per herd. Estimates of individual animal risks obtained by modeling within-herd correlation as overdispersion or random effects or even a combination of random and spatially dependent effects, as in the present study, do not suffer from this problem.

The region of the United States with the lowest herd seroprevalence of paratuberculosis in a previous study[5] was the south-central region, which only included Texas. However, it is not clear in that study what regions of Texas herds were selected from. Thus, it is possible that herds in west Texas were overrepresented, compared with the distribution of herds in the present study. It is also possible that this previous study did not include herds of purebred or Brahman cattle to the extent that the present study did. Both breed and location were risk factors for seropositivity in the present study, and differences in breed and location distributions may explain why our results appear so different from results of this previous study.

The sampling strategy we employed was subject to bias in that we were dependent on the willingness of owners to participate and could not randomly select herds for inclusion. One could suggest that ranchers who knew or suspected that they had paratuberculosis in their herd would be more likely to be willing to take advantage of the free testing associated with our study. On the other hand, ranchers whose livelihood depends on the sale of breeding stock may have been unwilling to test for fear of loss of reputation or the increased liability that follows documentation of the disease in the herd. We heard both sentiments expressed in conversations with potential participants and their veterinarians but were unable to determine which, if either, bias was predominant or affected our findings.

In the present study, spatial analysis showed an uneven distribution of seropositivity that was independent of species and water source effects. There was an uneven distribution of the test herds throughout the state because the concentration of cattle is not evenly distributed and probably because the concern about paratuberculosis is not as great in the western half of the state where the risk of infection is perceived to be lower. Despite the limitations and biases of the sampling strategy, there was a significant spatial risk pattern.

Several risk factors associated with seropositivity were identified in the present study. The increased risk associated with previous occurrence of clinical signs of paratuberculosis in the herd was no surprise. The history of having a dairy-type nurse cow was not unexpected by the investigators. In fact, we expected use of dairy-type cows as embryo transfer recipients to be a significant risk factor as well, but it was not. The prevalence of paratuberculosis is greater in dairy cattle than it is in beef cattle, and the practice of using cull dairy cows as nurse cows and embryo transfer recipients may lead to introduction of paratuberculosis into beef herds.

Having running streams as a source of drinking water was associated with increased odds of being seropositive. A running water source into which runoff from an infected herd flows could serve as a source of infection to a herd. On the other hand, ponds could serve as a source of infection within a herd leading to higher seroprevalence and increased probability of detection in a study such as the present one. Because

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only 37 herds in the present study had running streams as a water source and 23 of these also had ponds and well water as a source of drinking water, our findings should be considered preliminary and more detailed study of water source as a risk factor for paratuberculosis is warranted.

One of the most intriguing findings in the present study concerned the increased risks associated with the Brahman breed or *Bos indicus* cattle. Cattle from *Bos indicus*-based herds were more than 17 times as likely to be seropositive as were cattle from *Bos taurus*-based herds, and cattle from interspecies-based herds were 3.6 times as likely to be seropositive as were cattle from *Bos taurus*-based herds. This relationship could not be explained by confounding farm management factors or spatial risk. The OR for interspecies-based herds represented a risk close to half the full species risk when modeled on the logit scale. This graduated risk and the magnitude of the OR provides considerable evidence that may be considered causal of an increased risk of seropositivity among *Bos indicus*-based herds. This relationship, to our knowledge, has not been previously reported, although a previous study[1] found that Brahman cattle were more likely to be seropositive than were Angus cattle. There are several possible explanations for the higher seroprevalence in cattle from *Bos indicus*-based herds. There could simply be a higher prevalence of infection that became established within the species many years ago and was perpetuated and amplified by the trading of cattle among purebred herds. Another explanation could be that *Bos indicus* cattle are more susceptible to infection. These explanations seem less likely because of the large number of seropositive animals in herds with no known clinical or microbiologic evidence of disease. On the other hand, Brahman cattle could be more resistant to infection, and the seroprevalence may indirectly reflect this successful immunologic response to infection. Finally, these cattle could be responding to a different organism in a way different from the way *Bos taurus* cattle respond. This explanation is supported by the fact that of the 5 herds in the present study with high seroprevalence but an absence of clinical or microbiologic evidence of disease, 4 were *Bos indicus*-based herds and 1 was an interspecies-based herd. The present study, however, was not designed to explain the discrepancies among serologic responses, mycobacterial culture results, and clinical history.

We were able to isolate MAP from a relatively low proportion (7.3%) of seropositive cattle in the present study. Estimates of the specificity of ELISAs used for detection of paratuberculosis range from 97% to 99.8%[8-14] with specificity of the commercial kit used in the present study reported as 97.6%.[8] A conservative estimate of the minimum specificity of the ELISA used in the present study can be obtained from results of a previous study[5] in which > 10,000 beef cattle were tested and only 0.4% were found to be seropositive. At worst, if no infected animals were tested, the specificity would be 99.6%.[5] Taking both of these studies into account, we reasoned that approximately 1.5% of ELISA results in the present study would be false-positive results. Given that approximately 3% of test results were positive (ie, 137 cattle), we would expect that approximately half these results would represent false-positive results (ie, 69 cattle) and the other half would represent true-positive results (ie, infected cattle). For dairy cattle, sensitivity of mycobacterial culture of a single fecal sample has been reported to be 36%, irrespective of ELISA results.[9] Therefore, we would have expected culture results to be positive for at least 36% (ie, 25 cattle) of truly infected cattle with positive ELISA results. In contrast, results of mycobacterial culture were positive for only 10 cattle. It seems possible that sensitivity of our culture technique was less than that reported previously. However, because the second laboratory that performed mycobacterial cultures in the present study was the same laboratory that reported the 36% sensitivity for mycobacterial culture, the lower apparent sensitivity in the present study was more likely a result of handling of the fecal samples prior to culture. All samples were frozen and thawed prior to the first culture attempt and refrozen and thawed again prior to the second culture attempt. Only 4 of the 10 samples that yielded positive culture results at the first laboratory yielded positive results at the second laboratory, suggesting that refreezing and thawing had a negative effect. The present study was not specifically designed to evaluate the effect of storage on MAP viability. However, the effect of freezing on viability of MAP has been reported in 2 small studies.[15,16] A 30% to 80% loss of viability was reported after 3 weeks of storage at –70°C with no further significant loss after 15 weeks.[15]

An alternative explanation for the low number of positive culture results in the present study is that there may have been an unusually high proportion of uninfected cattle with positive serologic responses. There were 9 herds in the present study in which ≥ 12% of animals were seropositive. Owners of 4 of these herds reported that paratuberculosis had previously been diagnosed in the herd, and MAP was isolated from at least 1 animal in each of these herds. Owners of the remaining 5 herds reported that paratuberculosis had never been diagnosed in their herd, and results of mycobacterial culture were negative for these 5 herds. During follow-up telephone conversations performed up to 2 years later by one of the investigators (AJR), owners of 4 of these 5 herds confirmed that paratuberculosis still had not been diagnosed in the herd and that they were not experiencing any disease problems that resembled paratuberculosis. Thus, we hypothesize that other mycobacterial organisms may be causing positive serologic results in certain herds. From this, it is also possible that an agent other than MAP that was responsible for some positive serologic reactions may explain the spatial risk of seropositivity and the risk associated with use of rivers and streams as a water source. Forty-seven of the seropositive cattle were from these 5 herds or slightly more than a third of the total number of seropositive cattle in the study. If most of these cattle were truly uninfected but seropositive because of a different organism, we would have expected fewer positive culture results. This possible phenomenon of low specificity appeared to be clustered in herds because 97% of ELISA results in the present study were negative. Therefore, overall specificity

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of the assay could have been no worse than 97%; yet, some herds had high proportions of seropositive animals in the absence of clinical or microbiologic evidence of the disease.

In conclusion, seroprevalence of paratuberculosis among purebred beef cattle herds in Texas was greater than that reported for beef cattle herds in the United States as a whole but in line with prevalence reported from other southern states. This could be attributable to breed or regional differences in infection rates or interference by cross-reacting organisms. Analyses of risk factors for seropositivity suggested that having running streams as a water source, use of dairy-type nurse cows, previous clinical signs of paratuberculosis, having *Bos indicus* rather than *Bos taurus* cattle, and ranching in certain areas of the state increased the risk of paratuberculosis. Veterinarians should be aware of these risk factors as well as the unexpected serologic results found in some herds.

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a. HerdChek *Mycobacterium paratuberculosis* ELISA antibody, Idexx Laboratories Inc, Westbrook, Me.
b. Herrold's egg yolk agar slants with mycobactin J and ANV, Becton Dickinson Biosciences, Sparks, Md.
c. Copies of the survey are available from the corresponding author on request.
d. WinBUGS, version 1.4, MRC Biostatistics Unit, Cambridge, UK.
e. ArcView GIS 3.2, Environmental Systems Research Institute Inc, Redlands, Calif.

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