ABSTRACT: A cross-sectional study was performed to determine the odds of having a positive paratuberculosis ELISA result if the dam was ELISA positive in Texas beef cattle, adjusted for individual and herd-level risk factors for seropositivity. Texas beef cattle (n = 2,621) were tested for paratuberculosis by using a commercial ELISA and microbiologic culture of feces for *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Pedigree data were collected to identify dam- and sire-offspring pairs. Bayesian mixed-effects logistic regression was used to estimate the odds of seropositivity associated with age, dam ELISA status, sire ELISA status, herd size, herd history of clinical paratuberculosis, within-herd seroprevalence, within-herd fecal MAP prevalence, and within-herd fecal non-MAP *Mycobacterium* spp. prevalence. Herd of residence was included as a random effect to account for the correlation of observations within the same herd. Statistically probable associations were observed between ELISA status and herd fecal MAP prevalence [OR (odds ratio) 1.28 per 1% increase; *P* < 0.001] and herd seroprevalence (OR 1.21 per 1% increase; *P* < 0.001). The association with dam ELISA status was small (OR 1.35) and not highly probable (*P* = 0.69). Results indicate that use of dam ELISA status to make culling decisions in beef cattle may not improve the success of paratuberculosis control programs. Alternative strategies may be more effective for reducing the odds of seropositivity.

Key words: Bayesian modeling, beef cattle, genetic epidemiology, Johne's disease, *Mycobacterium avium* ssp. *paratuberculosis*

INTRODUCTION

Paratuberculosis is a chronic enteritis of ruminants associated with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection. Control programs in US beef herds have focused primarily on culling of clinically affected animals, management changes to reduce environmental contamination and calf exposure to MAP, and screening to identify subclinically infected animals (USDA, 2002). A common diagnostic method used in control programs is detection of serum antibody by using ELISA. These tests are favored because of the rapid availability of results when compared with fecal culture, low cost, and relative ease of sample collection. However, commercially available ELISA have been reported to have low sensitivity (Sackett et al., 1992; Whitlock et al., 2000; Dargatz et al., 2001; Collins et al., 2005; McKenna et al., 2005) and variable specificity in some herds (Osterstock et al., 2007; Roussel et al., 2007).

In light of evidence supporting increased risk of seropositivity in offspring of seropositive animals (Aly and Thurmond, 2005; Osterstock et al., 2008a), risks associated with in utero infection (Seitz et al., 1989; Sweeney et al., 1992b), and reported genetic associations (Koets et al., 2000; Nielsen et al., 2002; Mortensen et al., 2004; Gonda et al., 2006; Gonda et al., 2007), a potential strategy for paratuberculosis control is to cull the offspring of clinically affected or seropositive animals, regardless of the test status of the offspring. The effectiveness of this control measure may be reduced if a substantial proportion of serological reactions are due to factors unrelated to MAP infection.

The objective of this cross-sectional study was to evaluate the association between paratuberculosis ELISA status of the dam and ELISA status of her offspring in beef cattle adjusted for individual and herd-level risk
factors. Describing this association will aid producers and veterinarians in implementing paratuberculosis control programs and making culling decisions.

MATERIALS AND METHODS

The research project was approved by the Clinical Research Review Committee, Texas A&M University, College of Veterinary Medicine and Biomedical Sciences.

Animals and Diagnostic Testing

Animal selection methods and diagnostic testing procedures have been described previously (Osterstock et al., 2008a,b). Briefly, 2 groups of beef cattle in central Texas were identified for paratuberculosis testing. Texas Longhorn breeders within a 241-km radius of College Station, TX, were surveyed from a list obtained from 1 of 2 Texas Longhorn breed directories. The survey instrument was delivered by mail and solicited information regarding herd size, history of paratuberculosis, and availability of pedigree data. All herds with greater than 15 animals 2 yr of age or older and all herds with a history of clinical paratuberculosis, animals with undifferentiated chronic diarrhea and BW loss, or paratuberculosis test-positive animals were selected for sampling. A second group of herds were identified for sampling based on diagnosis of paratuberculosis within the herd through admission of individual animals to the Texas Veterinary Medical Center or by referral from practicing veterinarians in Texas. These herds were selected independent of beef cattle breed or availability of pedigree records. Pedigree data, when available, were collected for all registered animals in both groups of herds from breed registry certificates or production records. Breeders of all herds selected for sampling agreed to participate in the Texas Voluntary Johne’s Disease Program and provided written consent for enrollment in the research project.

All animals ≥2 yr of age within selected herds were tested for paratuberculosis. Blood samples were collected from the coccygeal or jugular veins into plain evacuated tubes (BD Vacutainer, BD Biosciences, Franklin Lakes, NJ). Fecal samples were collected from the rectum and were stored in individual plastic bags (Whirl-Pak, Advantec MPS Inc., Dublin, CA). Samples were shipped to a commercial laboratory (Johne’s Testing Center, Madison, WI) within 24 h of sample collection. Serum was harvested from collected blood samples and analyzed for MAP antibody by using a commercially available ELISA kit (HerdChek, IDEXX Laboratories Inc., Westbrook, ME). Serum ELISA results were converted to S:P (sample-to-positive control) ratios by taking the difference between the sample optical density (OD) and the mean of duplicate negative control OD and dividing by the difference between the means of the positive and negative control OD. Individual sample results were dichotomized into positive or negative by using the S:P ratio cutoff of 0.25, as recommended by the manufacturer. Fecal samples were processed for radiometric culture as described previously (Collins et al., 1990). Briefly, the medium was supplemented with mycobactin J, egg yolk suspension, and antimicrobials. Fecal samples were decontaminated with 1.0% hexadecylpyridinium chloride and concentrated via filtration. The filter membrane was subsequently placed into radiometric culture medium (Bactec 12B medium, BD Diagnostic Systems, Franklin Lakes, NJ) and evaluated weekly for growth by using an ionization detector (Bactec 460, Johnston Laboratories, Towson, MD). A PCR assay for the IS900 gene insertion element was used to identify MAP when acid-fast organisms were cultured. Mycobacterial isolates negative for IS900 were classified as non-MAP Mycobacterium spp. and further characterization was not performed.

Data Analysis

Pedigree and production records were used to verify age and identify dam-offspring and sire-offspring pairs for which both animals in the respective pair had available diagnostic test results. Animals from herds without sufficient production records to verify age or parent ELISA status were assigned missing values for these variables. Missing ELISA status of the sire and dam was imputed by using random assignment from a Bernoulli distribution with a success probability based on the individual animal-level prevalence of 3% that was reported in a similar beef cattle population when using the same ELISA kit (Roussel et al., 2005). Missing values for age were randomly assigned by using the distribution of observed ages in the sample population defined by using commercially available software for fitting distributions to data (@Risk, Palisade Corp, Ithaca, NY). Within-herd seroprevalence, fecal prevalence of MAP, and fecal prevalence of non-MAP Mycobacterium spp. were calculated from collected specimens and available diagnostic test data.

Individual paratuberculosis antibody status was modeled by using Bayesian mixed-effects logistic regression and Markov chain Monte Carlo (MCMC) techniques with available software (WinBUGS version 1.4, Medical Research Council, Cambridge, UK). For the purposes of this study, the primary exposure of interest was the ELISA status of the dam. Additional individual-level covariates selected for modeling included age of the animal and ELISA status of the sire. Herd-level covariates selected for modeling included herd size, herd history of animals with clinical paratuberculosis, ELISA seroprevalence, MAP fecal prevalence, and non-MAP Mycobacterium spp. fecal prevalence. Herd history of clinical paratuberculosis was defined as the presence of an animal during the 2-yr period before sampling with clinical signs of infection (BW loss and diarrhea) and diagnosed with paratuberculosis by a veterinarian. Herd of residence was included as a random effect to account for the correlation of observa-
Table 1. Priors for regression coefficients used in a model to estimate the association between paratuberculosis test status of the dam and her offspring in Texas beef cattle

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Dam ELISA status</td>
<td>0.23</td>
<td>1.15</td>
</tr>
<tr>
<td>Sire ELISA status</td>
<td>−36.28</td>
<td>3.90</td>
</tr>
<tr>
<td>Herd non-MAP(^2) Mycobacterium spp. status(^3)</td>
<td>0.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Herd MAP(^2) prevalence(^3)</td>
<td>25.77</td>
<td>11.55</td>
</tr>
<tr>
<td>Herd seroprevalence</td>
<td>18.36</td>
<td>2.50</td>
</tr>
<tr>
<td>Herd history of paratuberculosis(^4)</td>
<td>−0.08</td>
<td>0.22</td>
</tr>
<tr>
<td>Herd size</td>
<td>0.0002</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\(^1\)Priors for regression coefficients derived using conditional logistic regression for individual-level covariates and logistic regression for herd-level covariates. All priors were modeled by using a normal distribution.

\(^2\)MAP = Mycobacterium avium spp. paratuberculosis.

\(^3\)Defined based on results of microbiological culture of feces.

\(^4\)Defined as cattle with clinical symptoms of paratuberculosis and confirmatory diagnosis within 2 yr of sampling.

RESULTS

Samples were collected from 2,621 animals ≥2 yr of age from 22 beef cattle herds in central and coastal Texas. Mean herd size was 119 animals and ranged from 14 to 948 animals. A definitive age was available on 569 animals, with a mean of 6.8 yr (SD 3.9, range 2 to 18 yr). Within the sample population, 2,540 had fecal culture results, including 11 (0.4%) animals culture positive for MAP and 116 (4.6%) animals culture positive for non-MAP Mycobacterium spp. Serum ELISA results were available for 2,616 animals, including 96 (3.7%) that were seropositive. Mean within-herd prevalence of MAP based on fecal culture was 0.5% (range 0 to 4.1%) and mean within-herd fecal prevalence of non-MAP Mycobacterium spp. was 8.0% (range 0 to 71.2%). Mean within-herd seroprevalence was 4.7% (range 0 to 15.4%). Pedigree records identified 157 and 56 dam-offspring and sire-offspring pairs, respectively,
Table 2. Coefficient estimates for the mixed-effects logistic regression model developed to estimate the association between paratuberculosis test status of the dam and her offspring in Texas beef cattle, adjusted for individual and herd-level covariates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean β</th>
<th>OR(^1)</th>
<th>Lower</th>
<th>Upper</th>
<th>P-value, 2-sided(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (increase 1 yr)</td>
<td>−0.04</td>
<td>0.96</td>
<td>0.87</td>
<td>1.06</td>
<td>0.49</td>
</tr>
<tr>
<td>Dam ELISA status</td>
<td>0.30</td>
<td>1.35</td>
<td>0.27</td>
<td>5.95</td>
<td>0.69</td>
</tr>
<tr>
<td>Herd non-MAP(^3) Mycobacterium spp. status(^4)</td>
<td>−0.41</td>
<td>0.66</td>
<td>0.34</td>
<td>1.30</td>
<td>0.23</td>
</tr>
<tr>
<td>Herd MAP(^3) prevalence(^5) (increase 1%)</td>
<td>24.76</td>
<td>1.28</td>
<td>1.20</td>
<td>1.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Herd seroprevalence (increase 1%)</td>
<td>19.01</td>
<td>1.21</td>
<td>1.18</td>
<td>1.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Herd size (increase 10 head)</td>
<td>0.0001</td>
<td>1.00</td>
<td>0.99</td>
<td>1.01</td>
<td>0.68</td>
</tr>
</tbody>
</table>

\(^1\)OR = odds ratios.
\(^2\)Two-sided P-values were derived for parameter estimates by estimating the proportion of the posterior density >0 and <0 for parameters with negative and positive means, respectively, and multiplying by 2.
\(^3\)MAP = Mycobacterium avium ssp. paratuberculosis.
\(^4\)Defined based on results of microbiological culture of feces.
\(^5\)Mycobacterium spp. status was classified as “positive” if the dam also was classified as “suspect” or greater.

for which ELISA status was known for each member of the pair. One of 8 dam-offspring pairs with ELISA-positive offspring had an ELISA-positive dam and 11 of 149 pairs with ELISA-negative offspring had ELISA-positive dams. None of the 3 sire-offspring pairs with ELISA-positive offspring had ELISA-positive sires and 2 of 53 pairs with ELISA-negative offspring had ELISA-positive sires. The proportion of seropositive dams and sires was 7.6 and 3.6%, respectively.

Convergence was achieved after 20,000 iterations, and parameter estimates were based on an additional 10,000 iterations after burn-in. Sire status was removed from the model after an initial sensitivity analysis. The posterior density was highly dependent on the prior density. A contingency table (data not shown) for dam and sire ELISA status demonstrated that no animals had a negative dam and positive sire or a positive dam and negative sire. The results of the sensitivity analysis for the priors associated with sire status were attributed to multicollinearity because of this correlation. Herd fecal non-MAP Mycobacterium spp. prevalence was not linear in the log odds when included as a continuous variable and was therefore modeled by using 2 levels (<5% and ≥5%). The 5% cutoff corresponds to the proportion of animals with non-MAP Mycobacterium spp. isolations from feces in herds with unusual proportions of ELISA-positive animals given the herd history of paratuberculosis (Roussel et al., 2007). This binary classification did not demonstrate oversensitivity to prior densities and was retained in the model.

A significant decrease in DIC was noted after removal of herd history of clinical paratuberculosis from the model. Confounding of the association between other predictors and offspring ELISA status was not detected after removal of herd history. Removal of other terms from the model did not result in a significant improvement in model fit. The final model included age, dam ELISA status, herd size, herd ELISA prevalence, herd fecal MAP prevalence, herd fecal non-MAP Mycobacterium spp. prevalence, and a random effect term for herd. Odds ratios and 95% credibility intervals were derived from the parameter estimates for individual and herd-level covariates (Table 2). Results of the sensitivity analysis using noninformative priors on all parameters in the final model did not identify substantial changes in means of the posterior densities (data not presented).

Statistically probable associations were identified for herd seroprevalence (OR 1.21 per 1% increase; 95% confidence interval 1.18 to 1.24) and herd fecal MAP prevalence (OR 1.28 per 1% increase; 95% confidence interval 1.20 to 1.37). Associations between age, herd size, and herd fecal non-MAP Mycobacterium spp. status were small and not highly probable at the P < 0.05 level. The odds of having a positive ELISA result were estimated to be 1.35 times greater for the offspring of dams with positive ELISA results, but this association was not highly probable (P = 0.69).

**DISCUSSION**

Estimating the association between paratuberculosis test status of the dam and her offspring in beef cattle would be beneficial in herds with existing paratuberculosis control and management programs. One of the impediments to the success of paratuberculosis control programs is the long period of time after infection before the onset of clinical signs or the ability to detect infection by using currently available diagnostic tests. The findings in this study demonstrate that within-herd prevalence of MAP in feces and MAP-associated antibodies are associated with increased odds of beef cattle testing positive for paratuberculosis when using a commercial ELISA. In contrast to previous reports (Osterstock et al., 2008a), a small (OR 1.35) and statistically nonprobable (P = 0.69) association was observed between ELISA status of the dam and ELISA status of her offspring. In the previous report, odds of being classified as “suspect” or greater when using a commercial ELISA were 5 times greater if the dam also was classified as “suspect” or greater compared with offspring of dams classified as “negative.” The cutoff used for that
ELISA to classify animals as “suspect” or greater corresponded to an S:P ratio ≥0.10 (Collins, 2002). However, the influence of herd-level environmental risk factors on this association was not evaluated other than to include herd of residence as a random effect to account for the correlation of observations within herd. The smaller estimate of effect in this study may be due to the greater ELISA S:P ratio cutoff used here (S:P ≥ 0.25) compared with that used in the previous study (S:P ≥ 0.10). Alternatively, the smaller effect of dam ELISA status in a model that included herd-level covariates may indicate that the observed association in the previous study reflected similarities in the shared environment of the dam and her calf rather than specific maternal risk factors.

A factor that limits interpretation of results from the present study with regard to paratuberculosis infection is that the exposure to non-MAP Mycobacterium spp. in the environment has been associated with false-positive serological reactions when using paratuberculosis ELISA (Osterstock et al., 2007; Roussel et al., 2007). It is likely, given the disparity between the number of animals with MAP isolated from feces and the number of animals with positive ELISA results, that some of the ELISA-positive cattle were not infected with MAP. An alternative approach would have been to use culture for MAP in feces to characterize paratuberculosis status. However, the prevalence of fecal shedding of MAP in beef cattle is very low and precluded this approach in the present study because of insufficient power. Additionally, the objective of this project was to characterize the nature of these associations with an ELISA test that is commonly used in paratuberculosis control programs in beef cattle herds. We controlled for herd-level exposure to these Mycobacterium spp. by including fecal prevalence in the model. However, fecal prevalence of non-MAP Mycobacterium spp. could not be modeled as a linear term, and inclusion of this variable as a binary exposure may not have adequately accounted for this effect. In the present model, herds with fecal non-MAP Mycobacterium spp. prevalence ≥5% had decreased odds of being ELISA positive, although not at a statistically probable level. This appears counterintuitive given the reported association between environmental mycobacteria and paratuberculosis ELISA results (Osterstock et al., 2007; Roussel et al., 2007). This discrepancy may be because inclusion of herd seroprevalence in the model reduces the estimated effect of herd fecal non-MAP Mycobacterium spp. prevalence. Alternatively, this may reflect differences in specific Mycobacterium spp. present within herds. Previous reports have demonstrated that the proportion of specific Mycobacterium spp. isolated varies among herds and that different Mycobacterium spp. are associated with varying effects on paratuberculosis ELISA status (Osterstock et al., 2007). Speciation of non-MAP Mycobacterium spp. isolates was not performed in this study, but if the isolates between herds varied with respect to the likelihood of causing false-positive ELISA results, this may have affected the association as measured in this model.

Theoretically, a more complete evaluation of the influence of genetic factors on the odds of paratuberculosis seropositivity would have been achieved if ELISA status of the sire could have been included in the model. Recent reports have identified genetic associations with paratuberculosis antibody status in dairy cattle (Nielsen et al., 2002; Mortensen et al., 2004; Gonda et al., 2006). Heritability estimates of paratuberculosis status range from 6 to 10.2% (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006) and evidence for a quantitative trait locus on Bos taurus chromosome 20 has been reported (Gonda et al., 2007). In the present study, a small number of offspring had serological data available for the sire, contributing to sensitivity to the specified prior distributions. There also was evidence of multicollinearity between sire and dam ELISA status. Further study using beef cattle populations with more complete sire information may be useful to better define this relationship. The limited number of observations with serological data for the dam may have influenced the effect of dam in the present model and provided insufficient power to detect a difference. However, a similar influence of the prior on the regression coefficient for dam ELISA status was not observed.

Bayesian methods are an alternative to frequentist approaches to data analysis, but the 2 methods share many similarities (Greenland, 2006). The Bayesian approach uses prior distributions reflecting previous evidence of the strength of an association. That prior is updated by the present data and, using Bayes’s theorem, is summarized by a posterior distribution that reflects the combined evidence of the prior and the data collected in the present study. One of the parallels between Bayesian and frequentist methodology is that the parameter estimates obtained when using uninformative or vague priors are similar, as would have been achieved by using maximum likelihood estimation. A distinct advantage of Bayesian methods is that interpretation of the posterior distribution is more intuitive. The interval between the 2.5th and 97.5th percentiles can be interpreted as the interval we can be 95% confident contains the true parameter value. One limitation of Bayesian approaches is that the posterior distributions may be very complex and difficult to compute. However, MCMC sampling methods that sample from the posterior distribution can accommodate this complexity.

In this model, we used prior information regarding seroprevalence to impute missing serological data for the sire and dam and the empirical sample distribution of age to impute missing values for age. Data for missing animal-level covariates were generated to allow estimation of associations between animal and herd-level covariates and ELISA status by using all observations. Restricting the analysis to those animals with complete observations could have biased the estimates.
of effect, particularly for herd-level covariates, because the distribution of missing values was not independent of herd of residence. For instance, purebred herds with available pedigree records were more likely to have observations for which sire or dam ELISA status was available. Further, restricting the analysis to the small number of animals with complete information including dam and sire ELISA status would have resulted in insufficient precision. This is a limitation of studying a mixed population of commercial cattle of unknown pedigree and purebred animals with available registry certificates. Sensitivity to the prior distributions used to impute missing values was not detected in the model described here.

Parameter estimates for informative prior distributions were derived from the observed data by using maximum likelihood estimation. It would have been preferable to use data from previous reports to derive priors for the parameters in the model. However, limited information is available regarding the associations between ELISA status and familial and environmental risk factors for paratuberculosis in beef cattle. Further, deriving priors from data used to construct the model may unduly influence precision of the parameter estimates and introduce bias because of underestimated variability.

An additional limitation of the methods used here is the difficulty in selecting the appropriate terms to include in the model. Model selection in mixed-effects models, particularly with missing data, is very sensitive to the measure of model fit applied and the level of hierarchy to which it is applied, and is computationally intensive (Celeux et al., 2006). For the present model, we used a derivation of DIC and interpreted model fit at the individual animal level. Alternative measures of model fit may have yielded different results.

On the basis of these results, beef cattle producers and veterinarians should not emphasize the serological status of the dam when making paratuberculosis control decisions within herds, particularly regarding the culling of untested animals. This is in contrast to previous reports (Koets et al., 2000; Nielsen et al., 2002; Mortensen et al., 2004; Osterstock et al., 2008a) in which the influence of the paratuberculosis status of the dam was deemed important in predicting the paratuberculosis status of offspring. The difference between the relative importance of the association between the dam and her offspring observed here in beef cattle and in previous reports in dairy cattle is unknown. Intuitively, the typically longer duration of contact between a cow and her calf in beef cattle operations would be expected to increase the effect of the dam on offspring ELISA status compared with dairy cattle operations, in which the calf has limited exposure to the dam. However, calves in beef cattle operations also have prolonged contact with the entire adult population and the adult herd environment during the period when they are most susceptible to infection (Larsen et al., 1975). Dairy calves are typically removed from the maternity pen shortly after birth and are managed in an environment physically separated from the adult herd. Therefore, management of dairy calves is typically less variable between calves on the same farm and among calves on different farms. The primary difference between calves may be the paratuberculosis status of the dam and exposure to MAP from the dam that may occur during the immediate postpartum period, including contamination of the udder or immediate environment with feces containing MAP. Further study is needed to determine the specific differences between beef and dairy calf management that contribute to the differences in risk of seropositivity associated with ELISA status of the dam.

**LITERATURE CITED**


