Factors associated with ELISA scores for paratuberculosis in an Angus-Brahman multibreed herd of beef cattle[1]

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ABSTRACT: Cow and calf genetic and environmental factors were evaluated for their association with ELISA scores for paratuberculosis in a multibreed population of beef cattle. The ELISA scores are a measure of the presence or absence of antibodies against *Mycobacterium avium* subsp. *paratuberculosis* in bovine serum. The linear mixed-model analysis used 352 ELISA scores from 238 cows: 51 Angus (A); 34 Brahman (B); 41 (¾ A ¼ B); 45 (½ A ½ B); 34 (¼ A ¾ B); and 33 Brangus (⅝ A ⅜ B). Cows were assumed to be unrelated. Year affected ($P < 0.001$) ELISA scores, but age of cow did not, which was expected to be significant because of the chronic progressive nature of this disease. Important regressions on fixed effects associated with cows were 1) a positive estimate of cow B breed effect (0.59 ± 0.24; $P < 0.017$), indicating an upward trend of ELISA scores toward 100% B cows; 2) a negative estimate for weight change from before calving (late November) to the date of the blood sample in May (−0.0062 $\pm$ 0.0019 score/kg; $P < 0.002$), indicating that poorer maintenance of cow weights was associated with higher ELISA scores; and 3) a positive estimate for days in lactation of cow on the date of the blood sample (0.0086 $\pm$ 0.0034 score/d; $P < 0.021$), indicating the production of larger amounts of antibodies against *Mycobacterium avium* subsp. *paratuberculosis* as lactation progressed. Relevant regressions on fixed effects associated with calves were 1) calf birth weight (−0.022 $\pm$ 0.010 score/kg; $P < 0.035$), and 2) calf gain from birth to the date of the cow blood sample (−0.0092 $\pm$ 0.0027 score/kg; $P < 0.001$). These estimates indicate that cows that produced lighter calves at birth and/or calves with slower preweaning growth tended to have greater ELISA scores. Although the sensitivity (percentage of infected animals detected) of ELISA was only 50%, these results suggest that subclinical paratuberculosis may be negatively affecting cows and their offspring. Factors identified as associated with ELISA scores could help producers with culling decisions related to paratuberculosis control and eradication in beef cattle.

Key words: beef, cattle, Johne's disease, multibreed, *Mycobacterium avium* subspecies *paratuberculosis*, paratuberculosis

INTRODUCTION

Paratuberculosis (Johne's disease) is a chronic enteric disease of ruminants caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (**MAP**) that produces considerable economic losses in cattle because of decreased production and subsequent death (Nielsen et al., 2002; Stabel et al., 2002). Paratuberculosis is not only chronic but is also currently incurable, and it often remains in a subclinical state for years (Cousens, 2004). Thus, it is important to diagnose the disease as early as possible to minimize its effect and to improve the success of herd control programs. Paratuberculosis can be diagnosed by detection of serum antibodies (serological tests), by identification of bacteria (bacteriological cultures), and by DNA probes (Stabel, 1998). One commonly used serological test to detect subclinical paratuberculosis by herd screening is ELISA. The ability of ELISA to detect infected animals is low (i.e., low sensitivity), but its ability to detect noninfected animals is high (i.e., high specificity). Nonetheless, because it provides a reasonable method of herd screening, ELISA alone or in combination with fecal culture is the most frequently used diagnostic test to identify subclinical paratuberculosis in herds of cattle (Harris and Barletta, 2001; Whittington and Sergeant, 2001). To improve the effectiveness of ELISA as a tool in prevention and control programs of paratuberculosis

in beef cattle, it is important to identify factors that might be associated with or influencing ELISA scores. Improving the effectiveness of ELISA is particularly relevant in Florida, where the apparent prevalence of paratuberculosis in beef cattle was recently estimated to be 7.4% (Keller et al., 2004). Thus, the objective of this study was the assessment of various genetic and environmental factors associated with MAP ELISA scores in 3-yr-old and older cows from an Angus-Brahman multibreed herd of beef cattle.

MATERIALS AND METHODS

Paratuberculosis Detection Procedure

Cows were tested with ELISA, an antibody detection method designed to detect the presence of antibodies to MAP in bovine serum. Blood samples were collected from the coccygeal vein of cows in late May of 2003 and 2004 using an 18-gauge, 3.8-cm needle and a blood collection tube. Samples were identified, placed on ice in an insulated container, and transported to the laboratory to be centrifuged (4,000 rpm, 10 min) and to separate the serum. Serum was then transferred to storage containers, identified and stored at −6.7°C.

Serum samples were evaluated by ELISA (50% sensitivity and 99% specificity; *Mycobacterium paratuberculosis* Antibody Test Kit; IDEXX Laboratories, Westbrook, Maine). This ELISA kit was expected to identify 50% of MAP-infected cows, and 99% of noninfected cows. Thus, if 20 of 100 animals in a herd were infected with MAP, the test would be expected to 1) correctly identify 10 (50%) as infected and 79 (99%) as noninfected, and 2) incorrectly classify 10 (50%) of the infected animals as noninfected and one (1%) of the noninfected animals as infected.

The assay was done according to the directions of the manufacturer. Briefly, a micro titration system in which MAP antigens are coated on 96-well plates was used. Serum samples were diluted in a diluent containing *Mycobacterium phlei* to remove cross-reacting antibodies. On incubation of the diluted sample in the coated well, antibodies specific to MAP formed a complex with the coated antigens. After washing away unbound materials from the wells, a horseradish peroxidase conjugate (enzyme-labeled antibovine immunoglobulin) was added to bind to immunoglobulins bound to the solid-phase antigen. In the final step of the assay, unbound conjugate was washed away, and colorless enzyme substrate composed of hydrogen peroxide and a chromogen was added to wells. The chromogen in the enzyme substrate reacted with the enzyme portion of the conjugate to produce color. Subsequently, color was measured spectrophotometrically (optical density, 650-nm filter). Optical density was directly proportional to the amount of antibody present in the test sample. The presence or absence of antibody to MAP was determined by the sample-to-positive (**S:P**) ratio for each sample, in which S = optical density of the sample—optical density of the negative control, and P = optical density of the positive control—optical density of the negative control. The positive control was standardized and represented a significant level of antibody to MAP in bovine serum. Serum samples with S:P of <0.25 were classified as negative for MAP antibodies, and those with S:P of >0.25 were considered positive. The ELISA S:P were transformed into 5 ELISA scores based on the S:P categorized by Collins (2002):

1) 0 = negative, for S:P from 0 to 0.09; antibodies to MAP were not detected;
2) 1 = suspect, for S:P from 0.10 to 0.24; low level of serum antibodies but above-normal background levels;
3) 2 = weak positive, for S:P from 0.25 to 0.39; low level of serum antibodies to MAP but above the standard cutoff for a positive test;
4) 3 = positive, for S:P from 0.40 to 0.99; moderate level of serum antibodies to MAP; and
5) 4 = strong positive, for S:P from 1.00 to 10.00; high level of serum antibodies to MAP.

Animals and Data

Animals were from the Angus-Brahman multibreed herd kept at the Beef Research Unit of the University of Florida. A total of 352 weights (measured in late November and in late May), BCS, days of pregnancy (determined in August), and ELISA scores (from blood samples collected in late May) were obtained from 238 cows: 51 Angus (**A**); 34 Brahman (**B**); 41 (¾ A ¼ B); 45 (½ A ½ B); 34 (¼ A ¾ B); and 33 Brangus (⅝ A ⅜ B), from 2003 to 2004. Thus, there was an average of 1.5 ELISA scores per cow. Only cows that remained in the herd during 2003 and 2004 were sampled twice. Cows culled in 2003 and cows calving for the first time in 2004 had only one sample. Calf birth weights and preweaning weights (late May) from the progeny (n = 352) of these cows also were collected. Cows and calves were produced using a diallel-mating strategy, where sires from all breed groups were mated to cows from all breed groups. Table 1 shows the numbers of cows and ELISA scores per breed-group of maternal grandsire × breed-group of maternal granddam combination. Cows from all breed group combinations were represented in the data set, except for those from the mating of (½ A ½ B) grandsires to B granddams. There were 36 sires and 69 maternal grandsires from all breed groups represented in the data set. At least one sire within each breed group was used for 2 yr to create connections across years. Cows were synchronized in March with a progesterone-releasing device (CIDR; Pfizer Animal Health, Hamilton, New Zealand) for 7 d, followed by an injection of $PGF_{2\alpha}$ (5 mL of Lutalyse; Pfizer Animal Health), artificially inseminated twice, then assigned to a natural service sire group for a period of 60 d. There was 1 natural service sire group per breed group of sire, for a total of 6 breeding groups. Calves were born from mid December to mid March.

Table 1. Number of cows and number of ELISA scores (in parentheses) by breed group of maternal grandsire × breed group of maternal granddam[1]

Breed group of maternal granddam	Breed group of maternal grandsire						
	Angus	¾ A ¼ B	Brangus	½ A ½ B	¼ A ¾ B	B	All
Angus	23 (34)	3 (5)	7 (10)	6 (7)	7 (11)	5 (7)	51 (74)
¾ A ¼B	5 (8)	6 (9)	7 (11)	7 (11)	8 (12)	8 (10)	41 (61)
Brangus	9 (16)	2 (3)	12 (16)	3 (6)	3 (4)	4 (5)	33 (50)
½ A ½ B	14 (23)	4 (7)	8 (13)	5 (9)	6 (11)	8 (13)	45 (76)
¼ A ¾ B	7 (9)	7 (10)	3 (5)	4 (5)	5 (5)	8 (11)	34 (45)
B	4 (7)	1 (2)	4 (6)	0 (0)	2 (3)	23 (28)	34 (46)
All	62 (97)	23 (36)	41 (61)	25 (38)	31 (46)	56 (74)	238 (352)

[1]A = Angus; B = Brahman.

Management and Feeding

Cows and calves were maintained on bahiagrass (*Paspalum notatum*) pastures throughout the year with free access to mineral supplementation (Lakeland Animal Nutrition, Lakeland, FL). Because the multibreed herd was under a paratuberculosis control and eradication program, during the calving season (mid December to mid March) and until late April, first-calf heifers and older cows were assigned to 2 groups according to their ELISA score: 1) a low-risk group (0 = negative, and 1 = suspect); and 2) a high-risk group (2 = weak positive, and 3 = positive). The feeding regimen was the same for both groups. Cows were supplemented with bahiagrass and bermudagrass (*Cynodon dactylon*) hay (55% to 58% TDN and 7.5% to 12% CP, DM basis), cottonseed meal (*Gossypium* spp.; 78% TDN and 44% CP, DM basis), and molasses (72% TDN and 4.5% CP, DM basis). Access to annual ryegrass (*Lolium multiflorum*) pastures was provided from February to April. In late April, cows were assigned to 6 natural service breeding groups (one sire per breed group), and kept in these groups until weaning time of calves (September). From late September to mid December, first-calf heifers were kept in 1 group, and older cows were assigned to 3 groups according to their BCS (3 to 4, 5, and 6).

Statistical Analyses

The ELISA scores were analyzed using single-trait mixed-model methodology (Henderson et al., 1959; Henderson, 1973, 1984) with a simplified multibreed model (Elzo and Wakeman, 1998; Koonawootrittriron et al., 2002) that accounted for additive and nonadditive genetic and environmental fixed and random effects. Fixed subclass effects were 1) year (2003 and 2004), and 2) age of cow (3, 4, and ≥5 yr of age). Fixed linear covariates were 1) B breed effect of cows as a function of their fraction of B alleles; 2) cow heterosis effect as a function of intralocus interbreed interactions between Angus and Brahman alleles; 3) weight change of cow between the last weight prepartum (late November) and the weight on the date of her blood sample for ELISA (late May); 4) days in lactation of cows on the date of the blood sample; 5) BCS of the cow on the date of the blood sample; 6) days pregnant at palpation (mid August); 7) birth weight of the calf; and 8) preweaning gain of the calf between birth and date of blood sample of its dam. Random effects were cow and residual. Cows were assumed to be unrelated. Thus, random effects of cows had mean of 0 and common variance of σ_d^2. Similarly, residual effects were assumed to have mean of 0 and common variance of σ_e^2. Preliminary versions of this model included 2- and 3-way interactions. Because none of the interactions was significant ($P \leq 0.05$), they were excluded from the final model.

Computations were performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) using the REML option to estimate the cow and residual variance components. Predictions of ELISA scores for individual cows were obtained using estimates of relevant fixed effects and predictions of random effects from the mixed-model analysis, and computed using option OUTPRED of the MODEL statement of procedure MIXED.

Cow variance accounted for genetic and permanent environment variation among cows for ELISA scores. Estimation of cow and residual variances permitted the estimation of repeatability for ELISA scores (ratio of cow variance divided by the sum of cow plus residual variance), a measure of expected similarity among multiple ELISA scores of the same cow.

Means of predicted ELISA scores were plotted against individual effects in the model to obtain a clearer understanding of their relationship. To accentuate trends, linear regression lines of mean predicted ELISA scores on individual effects were added to each figure. Figures, regression lines, and regression equations were generated using the GPLOT procedure of SAS.

Control and Eradication

Except for 42 heifers introduced between 1993 and 1998, the Angus-Brahman multibreed herd used for this study has been maintained as a closed herd since 1988. Natural service sires have been exposed to this herd during the natural service breeding period.

Table 2. Unadjusted mean (SD) of ELISA scores of cows by breed group of maternal grandsire × breed group of maternal granddam[1]

Breed group of maternal granddam	Breed group of maternal grandsire						
	Angus	¾ A ¼ B	Brangus	½ A ½ B	¼ A ¾ B	B	All
Angus	0.62 (0.89)	0.80 (1.30)	1.00 (1.33)	0.86 (1.21)	0.55 (1.04)	1.43 (1.51)	0.77 (1.09)
¾ A ¼ B	0.50 (0.93)	0.89 (1.27)	1.00 (1.14)	0.36 (0.50)	0.58 (0.90)	1.20 (1.32)	0.74 (1.03)
Brangus	0.50 (0.89)	1.00 (1.00)	1.00 (1.21)	0.50 (0.84)	0.75 (1.05)	0.80 (1.30)	0.74 (1.07)
½ A ½ B	0.91 (1.08)	0.86 (0.69)	0.77 (1.01)	0.22 (0.44)	1.36 (1.03)	1.08 (1.04)	0.89 (0.99)
¼ A ¾ B	0.67 (0.87)	1.00 (1.41)	0.86 (0.84)	0.80 (1.30)	2.40 (1.14)	1.82 (1.54)	1.29 (1.32)
B	0.71 (1.11)	3.00 (1.41)	1.20 (1.17)	—	1.00 (1.00)	1.29 (1.18)	1.24 (1.20)
All	0.67 (0.94)	1.03 (1.23)	0.97 (1.11)	0.50 (0.83)	1.00 (1.15)	1.30 (1.26)	0.91 (1.12)

[1]A = Angus; B = Brahman.

Clinical cases of MAP infection have been observed at a low prevalence (1 to 2 cases per year) in this herd since 1988. There were 3 confirmed clinical cases of paratuberculosis in 2003 and 4 in 2004. Cases were confirmed based on clinical presentation, gross pathology, and histopathological evaluation. Consequently, annual risk assessment and management plans were formulated to decrease microbiological contamination and spread of the organism (USAHA-NJWG, 2003a,b). Most important to the management plan was the decrease in exposure of calves and young stock to shedding cows and their manure. This effort included implementation of specific management practices, additional serological and/or fecal testing, and removal of animals that were either positive or highly likely to be infected. Specific management practices implemented were 1) use of hay rings and their regular movement in the pasture during winter feeding to decrease feed (and bedding) contamination, 2) use of water troughs constructed to minimize fecal contamination, and 3) separation of prepartum cows by age and paratuberculosis exposure status based on the herd ELISA screening test. In addition, cows that showed clinical signs of paratuberculosis were further tested by ELISA and/or PCR test. Those that were positive or had a high likelihood of being infected with paratuberculosis (score 4 = high positive) were separated from their herd mates, kept in a quarantine pasture, and then removed from the herd together with their calves; these calves were sent to a terminal market, and cows were sent to slaughter.

RESULTS AND DISCUSSION

Data

The Angus-Brahman multibreed herd was designed to be a microcosm of the type of cattle present in Florida. The diallele-mating strategy has produced animals with a variety of A and B breed compositions that could potentially yield a large variation in intensity of serum antibody responses as measured by ELISA if this response differs between these 2 breeds. Table 2 presents unadjusted means and SD of ELISA scores by breed-group of maternal grandsire × breed-group of maternal granddam subclass. Maternal grandsires and maternal granddams are the parents of cows and maternal grandparents of calves. Means suggest that cows produced by all mating combinations had some degree of antibody reaction to MAP and that this reaction seemed to increase with the fraction of B in the cow. The majority of SD was larger than the means, which indicated the existence of a sizable amount of variation among cow ELISA scores within breed-group of maternal grandsire × breed-group of maternal granddam subclasses. Probably largely because of small numbers per subclass, mean scores by individual maternal grandsire breed groups mated across maternal granddam breed groups (columns in Table 2) showed a less clear trend to increase with a larger B fraction in the progeny of these matings than the mean scores by individual maternal granddam breed groups mated across maternal grandsire breed groups (rows in Table 2). Nonetheless, overall mean scores per maternal grandsire group (last row in Table 2) and overall mean scores per maternal granddam group (last column in Table 2) showed a clear upward trend from A to B. This trend was even more evident in the mean scores (±SE) by breed group of cow: 0.69 ± 0.12 for A, 0.80 ± 0.12 for (¾ A ¼ B), 0.83 ± 0.12 for (½ A ½ B), 1.36 ± 0.17 for (¼ A ¾ B), and 1.40 ± 0.20 for B. The mean for Brangus cows (0.72 ± 0.15) was similar to that of A cows. A particular breed group of cow included all cows of a given breed composition regardless of the breed composition of their parents. For example, (¾ A ¼ B) cows were produced by the mating of (½ A ½ B) maternal grandsires × A maternal granddams, (¾ A ¼ B) maternal grandsires × (¾ A ¼ B) maternal granddams, and A maternal grandsires × (½ A ½ B) maternal granddams (Table 1). Greater ELISA scores are related directly to likelihood of infection with MAP (Collins, 2002); however, greater levels of antibodies against MAP could indicate either greater susceptibility to infection or greater resistance to infection, as first noted by Roussel et al. (2002). Unadjusted means indicate that animals of different Angus and Brahman fractions may have different immunological responses to MAP.

Table 3. Generalized least square estimates of fixed effects in the model for ELISA scores

Effect	Estimate	SE	$P > |t|$
Yr 2003[1]	1.86	0.66	0.006
Yr 2004[1]	1.34	0.66	0.045
3-yr-old cows[2]	−0.13	0.14	0.34
4-yr-old cows[2]	0.13	0.14	0.35
Brahman fraction of cow[3]	0.59	0.24	0.017
Heterosis of cow	0.05	0.28	0.85
Weight change of cow from November to May, score/kg	−0.0062	0.0019	0.002
Days in lactation of cow, score/d	0.0086	0.0034	0.021
BCS of cow	−0.049	0.077	0.52
Days pregnant of cow, score/d	0.0000	0.0014	0.98
Birth weight of calf, score/kg	−0.022	0.010	0.035
Preweaning gain of calf from birth to May, score/kg	−0.0092	0.0027	0.001

[1]Expectation of solution for year effect includes the mean.
[2]Age of cow effects are deviated from 5-yr-old and older cows.
[3]Brahman breed effects are deviated from Angus.

Mixed-Model Analysis

Results are presented and discussed using estimates of fixed effects, predictions of random effects, and figures of mean predicted ELISA scores plotted against relevant effects in the model.

Table 3 shows the generalized least squares estimates for fixed effects. Solutions for year effects include the overall mean; those for 3- and 4-yr-old cow effects are expressed as deviations from ≥5-yr-old cows; and all other solutions are estimates of their respective effects in the model.

Subclass Fixed Effects. The ELISA scores were affected by year ($P < 0.001$) but not by age of cow, which was unexpected because paratuberculosis is a chronic progressive disease that would be expected to yield larger ELISA scores in older than in younger cows. Possible explanations are that 1) cows may not be getting infected as calves but mostly later in life; 2) the speed of progress of the disease was too variable within age of cow subclasses, perhaps related to differences in infective dose and/or immune response among cows, thereby masking age of cow effects; 3) the sensitivity of ELISA (50%) was too low to permit an accurate assessment of the stage of the disease; and 4) the data set was too small to obtain significant differences among cow ages.

Regression on Fixed Effects Associated with Cows. Several characteristics of cows and their calves were found to be good predictors of ELISA scores for paratuberculosis. Cow B breed effects (deviated from A) were an important factor (0.59 ± 0.24; $P < 0.017$). Mean predicted ELISA scores showed a distinct upward trend from A to B cows (Figure 1). Predicted means from cows that were 75% B and greater were more variable than those from A cows and less than 75% B cows. Assuming a similar opportunity for all animals in the multibreed to become infected, the positive value of the (B − A) estimate could be either an indication that animals with greater B fractions would be more susceptible or more resistant to paratuberculosis (Rous-

sel et al., 2002). This discussion should be tempered by the fact that this research does not permit detection of cause-effect relationships. Other factors associated with the A and B breeds also could affect these results (e.g., behaviors such as eating and nurturing habits) or, as suggested by Roussel et al. (2002), *Bos indicus* cattle might be responding to bacteria other than MAP differently from *Bos taurus* cows. Nonetheless, it is unlikely that there were cross-reacting organisms here because the Angus-Brahman multibreed herd had a common level of antigen exposure in the environment.

Cow heterosis, measured as intralocus interbreed interaction effects, was not an important factor for ELISA scores. However, the negative regression (-0.0062 ± 0.0019; $P < 0.002$) of ELISA score on change in weight experienced by cows between the date of the last precalving weighing (late November) and the date of the blood sample (late May) indicates that ELISA scores were greater in cows that were less capable of maintaining BW during this period. This negative association between weight change of cows and ELISA scores can be clearly seen in Figure 2. The larger the weight
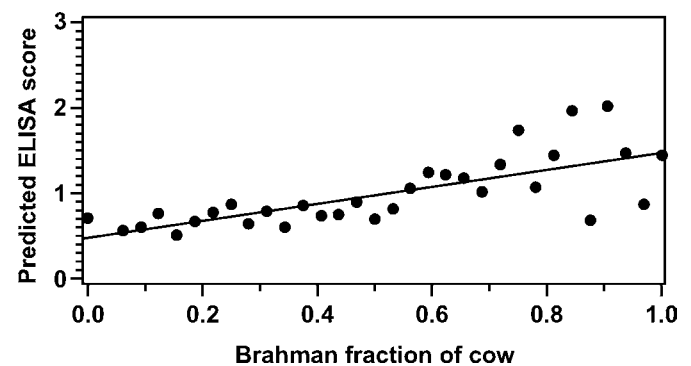
Figure 1. Mean predicted ELISA scores by Brahman fraction of cow. The linear regression of mean predicted ELISA scores on Brahman fraction of cow was $0.48 + 1.00 \times$ Brahman fraction of cow.
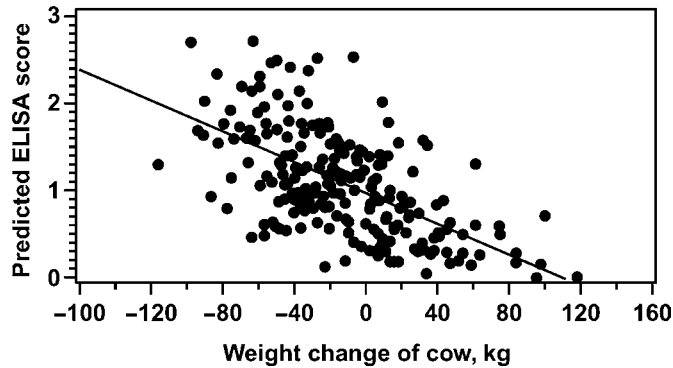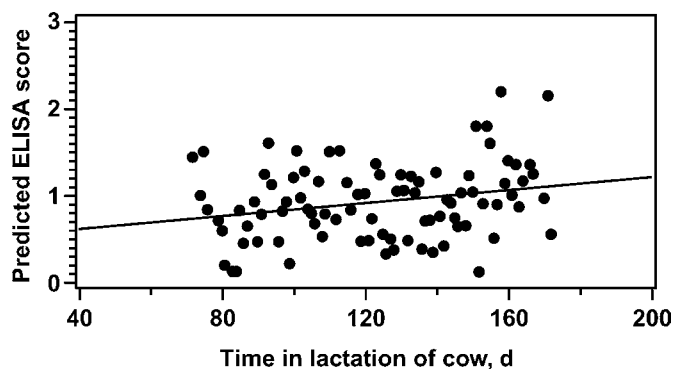
Figure 2. Mean predicted ELISA scores by weight change of cow between November and May weights. The linear regression of mean predicted ELISA scores on weight change of cow was $0.97 - 0.0088 \times$ weight change of cow.

Figure 4. Mean predicted ELISA scores by days pregnant of cow. The linear regression of mean predicted ELISA scores on days pregnant of cow was $1.54 - 0.005 \times$ days pregnant of cow.
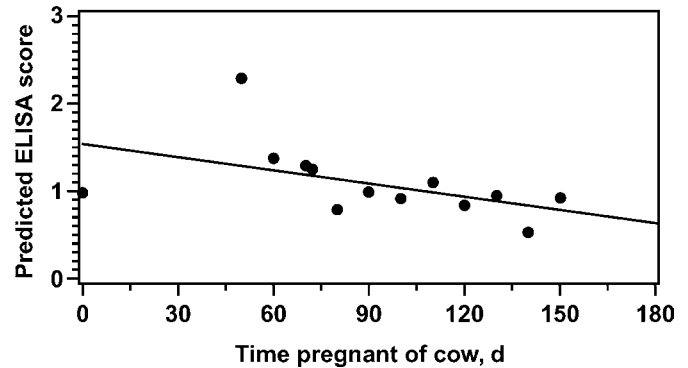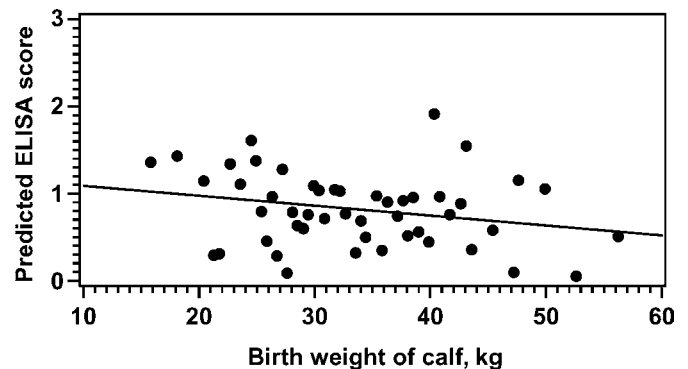
loss (if negative) and the less gain in weight (if positive), the greater the mean predicted ELISA score.

Blood samples of cows were taken between the fourth and the sixth month of their lactation depending on calving date. Thus, the positive association that existed between days in lactation of cows on the date of the blood sample and ELISA scores (regression of ELISA scores on days in lactation = 0.0086 ± 0.0034 score/d; $P < 0.021$; Figure 3) could be an indication of increased antibody response against MAP as demand for resources allocated to milk production decreased during the second half of the lactation.

Neither BCS nor days pregnant of cow on the date of blood sample collection was significantly associated with ELISA score. The plot of mean predicted ELISA scores against BCS of cows had a small downward slope in agreement with the nonsignificant value of the regression estimate for this effect. However, the graph of the mean predicted ELISA scores vs. days pregnant of cows showed a clear downward trend (Figure 4), unlike the regression coefficient of 0 estimated for days preg-

nant in the mixed-model analysis. The small size of the data set used here may have prevented this effect from achieving significance.

Regression on Fixed Effects Associated with Calves. There were negative regression estimates of ELISA scores of cows on birth weight of calf (-0.022 ± 0.010 score/kg; $P < 0.035$) and on preweaning gain of calf from birth to the date of the blood sample of its dam in May (-0.0092 ± 0.0027 score/kg; $P < 0.001$). Cows that gave birth to lighter calves tended to have greater ELISA scores than cows with heavier calves at birth (Figure 5). Similarly, cows whose calves had smaller preweaning gains tended to have greater ELISA scores than cows whose calves had greater preweaning gains (Figure 6). These results may be an indication that cows with subclinical paratuberculosis provided a lower level of nutrition to the fetus and were unable to produce as much milk as uninfected cows, and, hence, the lower birth weights and lower preweaning gains of calves.

Variance Due to Cow Random Effects. Cows were assumed to be unrelated in the model used here. Be-

Figure 3. Mean predicted ELISA scores by days in lactation of cow. The linear regression of mean predicted ELISA scores on days in lactation of cow was $0.48 + 0.0037 \times$ days in lactation of cow.

Figure 5. Mean predicted ELISA scores by birth weight of calf. The linear regression of mean predicted ELISA scores on birth weight of calf was $1.19 - 0.011 \times$ birth weight of calf.
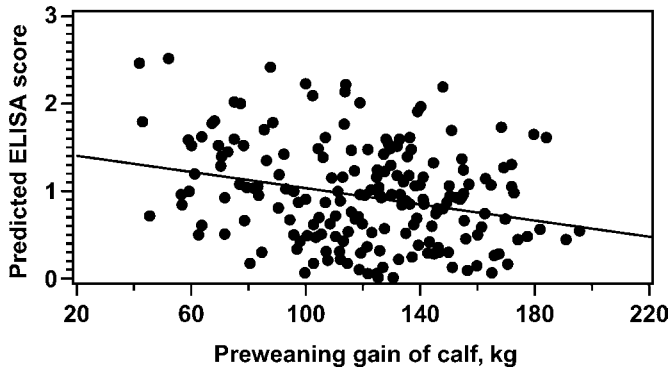
Figure 6. Mean predicted ELISA scores by preweaning gain of calf. The linear regression of mean predicted ELISA scores on preweaning gain of calf was $1.50 - 0.0046 \times$ preweaning gain of calf.

cause some cows were related, the genetic portion of the cow variance may have been underestimated because similarity among related cows was unaccounted for; however, estimates of heritability for antibody response have been small (<10%) in dairy cattle (Nielsen et al., 2002; Mortensen et al., 2004). Thus, because of the sizable number of sires of cows (n = 69) and the likely small cow genetic variation for ELISA scores present in this data set, underestimation, if it occurred, was probably small. The REML estimate of the variance due to cows was 0.34 ± 0.11, and that of residual was 0.65 ± 0.09. The estimate of repeatability (ratio of cow variance divided by the sum of cow and residual variances) was 0.34 ± 0.01. The SE of the repeatability estimate was computed using the delta method (MacIntosh and Hashim, 2003). The estimates of the cow variance and of repeatability indicate considerable variation in ELISA scores among cows and that scores for cows were fairly repeatable across years. Such estimates need to be interpreted with caution because this study involved only 2 years of data, so the number of ELISA scores per cow was at most 2.

Control and Eradication. Current control and eradication programs for paratuberculosis are based on detection of MAP in the animal and in the environment and on management strategies to minimize contamination and spread of the disease. Currently, age of cow is the only animal factor considered in the management strategies. The present research, however, suggests that other animal factors could be of potential use during the subclinical phase of paratuberculosis to improve control and eradication strategies, as well as to help diagnose MAP. Control measures could include breed group of cows and weight changes of cows to categorize cows within a herd in addition to age of cow. Traits of the calf also could be used to create alternate categories for grouping cows. For example, prepartum cows could be grouped by age × breed group × ELISA score subclass. Postweaning cows could be grouped by age × breed group × ELISA score × preweaning calf growth subclass,

or by age × breed group × ELISA score × weight change of cow. The assumption is that the significant cow and calf effects identified here would improve our ability to discriminate among cows that are actually infected with paratuberculosis among those tested by ELISA. Effects such as the cow and calf effects discussed here could eventually be used to aid in prediction of the stage of subclinical paratuberculosis in individual animals; however, this last aspect would require precise knowledge of the infection status of each cow. Recent studies have shown that DNA techniques (Amonsin et al., 2004; Vansnick et al., 2004) could eventually be used as practical tools to identify infected animals. If infected cows were known, regression factors such as the ones identified here could be used as indicators of susceptibility to paratuberculosis or to help predict the stage of the disease in individual animals. Consequently, 1) more precise control and eradication strategies could be designed by using a priori knowledge of expected susceptibility and speed of progression of the disease in specific groups of animals, and 2) more accurate and possibly earlier identification of animals that would likely need to be culled to curtail the spread of paratuberculosis within herds could be made.

Final Remarks. In pasture-based cow-calf management systems, it is difficult to break the infection cycle of a bacterium such as MAP that is spread through feces. This fact, coupled with inaccurate procedures to identify the infectious status of individual animals, provides MAP ample opportunity to propagate and become more severe in a herd over time. Paratuberculosis is still considered primarily a disease of dairy cattle. Most beef cattle producers have ignored this disease as a less common, occasional robber of productivity of mature cows. Apparent herd prevalence for paratuberculosis in beef cattle has ranged from 3% to 8% in various states of the US (Thorne and Hardin, 1997; Roussel et al., 2002; Hill et al., 2003). For beef cattle in Florida, apparent prevalence has changed little in the last 14 yr: 8.6% in 1990 (Braun et al., 1990) and 7.4% in 2004 (Keller et al., 2004). The apparent prevalence in the multibreed beef cattle herd used in this study was 35.1%, almost 5 times greater than the prevalence reported by Keller et al. (2004). This herd, apart from the breeding strategy, has been managed as a commercial beef cattle operation. No test for paratuberculosis was required of animals entering the herd before 2003; thus, the number of clinical cases of paratuberculosis increased until that year. Other beef cattle operations using similar animal introduction strategies could see a dramatic increase in the prevalence of this disease. Consequently, if the prevalence of paratuberculosis in beef herds is to be kept low, all beef cattle herds would need to implement paratuberculosis control and eradication procedures.

Paratuberculosis studies in beef cattle have not considered its possible influence on cow production and calf performance. Results of the current study suggest that such a relationship very likely exists and that the

effect extends well beyond the loss of a cow in the latter stages of this disease.

IMPLICATIONS

Paratuberculosis is an incurable chronic disease that produces sizable economic losses in dairy cattle. Less information is available on the effect on beef cattle, but prevalence seems to be slowly increasing. High ELISA scores were associated with decreased cow weights, smaller calf birth weights, and decreased preweaning gains, possibly as a result of negative effects of paratuberculosis infection on cow milk production. Although the ELISA test we used may have low diagnostic sensitivity, it seems to detect the stage of paratuberculosis infection in beef cattle when production effects of the disease are evident. The association of ELISA scores with cow Brahman fraction could be related to increased susceptibility or increased resistance of this breed to paratuberculosis or simply reflect a breed effect on level of antibody production in response to infection. The results reported for this study need to be reconfirmed with larger samples of beef cattle and for a variety of breeds.

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