

Factors associated with ELISA s/p ratio scores for paratuberculosis in an Angus-Brahman multibreed herd of beef cattle

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SUMMARY

Dam and calf genetic and environmental factors were evaluated for their association with enzyme-linked immunosorbent assay (ELISA) s/p ratio scores for paratuberculosis in a multibreed beef cattle population. The linear mixed model analysis used 359 ELISA s/p ratio scores from 238 dams : 51 Angus (A), 34 Brahman (B), 41 (% A % B), 45 (% A % B), 34 (% A % B), and 33 Brangus (5/8 A 3/8 B). Dams were assumed to be unrelated. The MIXED procedure of SAS was used to perform computations. Year affected ELISA s/p ratio scores, but not age of dam, which was expected to be significant because of the chronic progressive nature of this disease. **Important dam regression effects were:** 1) positive B – A effect indicating an upward trend of ELISA scores towards 100% B dams, 2) negative dam weight change from before calving until May indicating a negative association between weight maintenance and ELISA scores, and 3) positive association with days in lactation indicating an improvement in antibody production against MAP as lactation progresses. **Relevant calf regression effects were:** 1) birth weight, and 2) calf gain from birth to the date of the dam blood sample. Dams with high ELISA s/p ratio scores produced smaller calves, gained less weight (or lost weight) during the preweaning season, and produced less milk, which in turn may have caused calves to have smaller preweaning gains. Factors identified here as associated with ELISA s/p ratio scores could help cattle producers with culling decisions related to paratuberculosis control and eradication efforts in beef cattle.

INTRODUCTION

Paratuberculosis is a chronic enteric disease of ruminants caused by the bacterium *Mycobacterium avium* subsp. paratuberculosis (MAP) that produces considerable economic losses in cattle due to reduced production and subsequent death. Paratuberculosis often remains in a subclinical state for years. Thus, it is important to be able to diagnose the disease as early as possible to minimize its impact and to improve the effectiveness of herd control programs. A commonly used serological test to detect subclinical paratuberculosis by herd screening is the enzyme-linked immunosorbent assay (ELISA). The ability of ELISA to detect infected animals is low (i.e., low sensitivity), but their ability to detect non-infected animals is high (i.e., high specificity). 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 may be influencing ELISA scores. This is particularly relevant in Florida where the true prevalence of paratuberculosis in beef cattle was recently estimated to be 13.1%.

The objective of this study was the assessment of various genetic and environmental factors associated with MAP ELISA s/p ratio scores in three year old and older cows from an Angus-Brahman multibreed herd of beef cattle.

MATERIAL AND METHODS

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 (late November and late May), condition scores, days of pregnancy (August), and ELISA scores were obtained from 238 cows: 51 Angus (A), 34 Brahman (B), 41 % A % B, 45 % A % B, 34 % A % B, and 33 Brangus (5/8 A 3/8 B) dams from 2003 to 2004. Thus, there was an average of 1.5 records per dam. Calf birth weights and preweaning weights (late May) from the progeny (n = 352) of these dams were also collected. Cows and calves were produced using a diallel-mating strategy where sires from all breed groups were mated to dams from all breed groups. Table 1 shows the numbers of dams and ELISA s/p ratio scores per breed-group-of-maternal grand sire + breed-group-of-maternal granddam combination. At least one sire within each breed group was used for two years to create connections across years. Cows were synchronized in March with a progesterone-releasing device (CIDR[®], Pfizer Animal Health) for 7 d, followed by an injection of PGF_{2α} (5 ml of LUTALYSE[®], Pfizer Animal Health), artificially inseminated twice, then assigned to a cleanup sire group for a period of 60 d. There was one cleanup sire group per breed group of sire, for a total of six breeding groups. Calves were born from mid-December to mid-March.

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

Paratuberculosis detection procedure. All cows were tested by the ELISA antibody detection method using blood samples collected in late May of 2003 and 2004. Samples were transported to the laboratory to be centrifuged and serum separated. Serum samples were evaluated for antibodies against MAP by ELISA (50% sensitivity and 99% specificity, IDEXX Laboratories, Mycobacterium paratuberculosis Antibody Test Kit). The presence or absence of antibody to MAP is determined by the sample to positive (s/p) ratio for each sample, where 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 following five ELISA s/p ratio scores were defined based on the s/p ratios categorized by Collins (2002):
 1) 0 = negative = s/p ratios from zero to 0.09; antibodies to MAP were not detected,
 2) 1 = suspect = s/p ratios from 0.10 to 0.24; low level of serum antibodies but above normal background levels
 3) 2 = weak positive = s/p ratios 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 = s/p ratios from 0.40 to 0.99; moderate level of serum antibodies to MAP, and
 5) 4 = strong positive = s/p ratios from 1.00 to 10.00; high level of serum antibodies to MAP.

Statistical analysis. The ELISA s/p ratio scores were analyzed using single-trait mixed model methodology with a simplified multibreed model 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 dam (3 yr, 4 yr, and 5 yr and older cows). Fixed linear covariate effects were: 1) dam B breed effect (deviated from A) as a function of the B fraction in dams, 2) dam heterosis effect as a function of intralocus interbreed interactions between Angus and Brahman alleles, 3) dam weight change between the last weight prepartum (late November) and the weight on the date of her blood sample for ELISA (late May), 4) dam days in lactation on the date of the blood sample, 5) dam condition score on the date of the blood sample, 6) days pregnant at palpation (mid-August), 7) birth weight of calf, and 8) calf preweaning gain between birth and the date of the dam blood sample. Random effects were dam and residual. Dams were assumed to be unrelated. Two and three-way interactions were non-significant, thus they were excluded from the final model.

Predictions of individual ELISA s/p ratio scores were computed using estimates of relevant fixed effects and predictions of random effects from the mixed model analysis. Computations were performed using SAS Proc MIXED with option REML to estimate variance components. Means of predicted ELISA s/p ratio scores were plotted against individual effects in the model using the GLOT procedure of SAS.

Table 2. Generalized least squares of fixed effects for ELISA s/p ratio scores

Effect	Estimate	SE	P > t
Dam Breed (Brahman – Angus)	0.5857	0.2410	0.0168
Maternal Heterosis	0.0536	0.2754	0.8461
Dam weight change from November to May	-0.0062	0.0019	0.0016
Dam days in lactation	0.0086	0.0034	0.0205
Dam condition score	-0.0492	0.0765	0.5217
Dam days pregnant	0.0000	0.0014	0.9817
Calf birth weight	-0.0215	0.0100	0.0348
Calf gain from birth to May	-0.0092	0.0027	0.0011

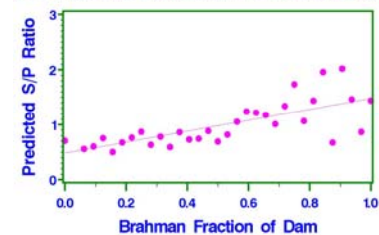
RESULTS AND DISCUSSION

Table 2 shows the generalized least square estimates for dam and calf regression fixed effects. Figures depict trends in mean predicted ELISA s/p ratio scores according to values of dam and calf continuous variables in the multibreed dataset.

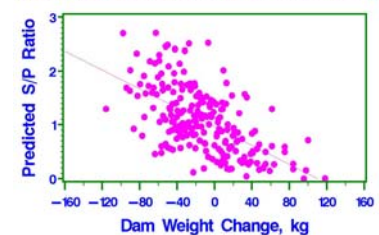
Dam regression fixed effects. The association between ELISA s/p scores was positive with dam B – A breed effects, negative with dam change in weight between the date of the last pre-calving weighing (late November) and the date of the blood sample (late May), and positive with days in lactation. Thus, higher levels of antibodies against MAP were produced by dams with higher B fractions. However, dams with high ELISA scores gained less weight (or lost weight). Dams produced more antibodies later in their lactations suggesting an improvement in their ability to react to MAP.

Neither dam condition score nor days pregnant on the date of the dam blood sample were significant. However, the graph of the mean predicted ELISA s/p ratios versus dam days pregnant showed a clear downward trend. Perhaps the small size of the dataset prevented this dam effect from achieving significance.

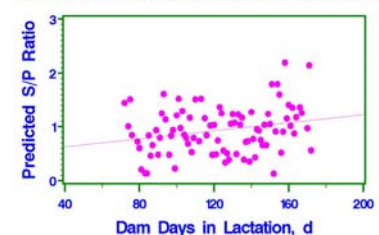
MEAN ELISA S/P RATIO SCORES BY BRAHMAN FRACTION OF DAM



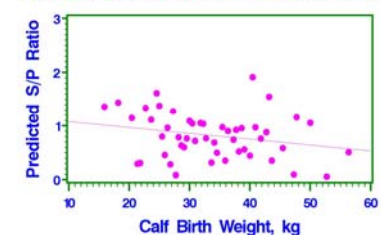
MEAN ELISA S/P RATIO SCORES BY DAM WEIGHT CHANGE



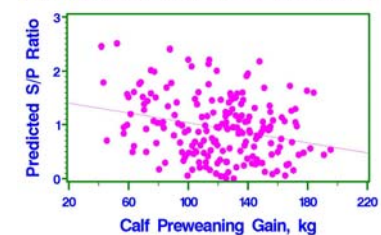
MEAN ELISA S/P RATIO SCORES BY DAM DAYS IN LACTATION



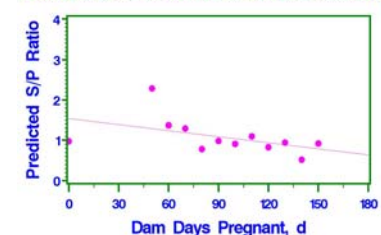
MEAN ELISA S/P RATIO SCORES BY CALF BIRTH WEIGHT



MEAN ELISA S/P RATIO SCORES BY CALF PREWEANING GAIN



MEAN ELISA S/P RATIO SCORES BY DAM DAYS PREGNANT



Calf regression fixed effects. There was a negative association between ELISA s/p ratio scores in dams with calf birth weight and calf gain from birth to the date of the dam blood sample in May.

Progeny from dams with higher ELISA s/p ratio scores had smaller birth weights and smaller preweaning gains. These results may be an indication that dams with subclinical paratuberculosis were unable to produce as much milk as uninfected dams, thus their calves tended to grow more slowly than progeny from uninfected dams.

Repeatability. The estimate of repeatability (ratio of dam variance divided by the sum of the dam plus the residual variance) was 0.34 ± 0.01. This repeatability estimate suggests that there was a sizable variation in the ELISA s/p ratio scores among dams, and that scores were fairly repeatable within dams across years.

Final Remarks. This study found high ELISA s/p ratio scores to be associated with lower cow weights as well as lower calf birth weight and lower preweaning gains, likely due to lower milk production. Thus, although ELISA tests have low sensitivity, they suggest that there may be a significant negative impact of subclinical paratuberculosis on production traits of dams and calves. Results here need to be reconfirmed with larger samples of beef cattle of a variety of breeds.