Short communication: Heritability estimates for susceptibility to *Mycobacterium avium* subspecies *paratuberculosis* infection defined by ELISA and fecal culture test results in Jersey cattle

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ABSTRACT

Paratuberculosis (Johne's disease), an enteric disorder in ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis*, causes economic losses in excess of \$200 million annually to the US dairy industry. Costly diagnostic testing, cumbersome control programs, incurability, and ineffective vaccination all make *M. avium* ssp. *paratuberculosis* susceptibility a good candidate for genetic studies and genetic selection a potentially useful adjunct to management-based control programs. No report has been published for heritability of susceptibility to *M. avium* ssp. *paratuberculosis* infection in Jersey cattle. The objective of this study was to estimate variance components and heritability for susceptibility to *M. avium* ssp. *paratuberculosis* infection in US Jersey cattle. Data consisted of complete serum ELISA and partial fecal culture results on a total of 2,861 Jersey cows from 23 commercial herds throughout the United States after editing. Four *M. avium* ssp. *paratuberculosis* susceptibility phenotypes were defined using (1) ELISA sample-to-positive ratios as a continuous trait, (2) ELISA results as a binary trait (positive = 1, negative = 0), (3) ELISA results as an ordered categorical trait, and (4) a combined test in which ELISA and fecal culture results were both taken into account in a binary analysis. Three statistical models, including linear, binary threshold, and ordered threshold sire models, were used to analyze the data. All analyses were executed using the restricted maximum likelihood method in AS-Reml 3 software. The heritability estimates were low to moderate and ranged from 0.08 (± 0.03) to 0.27 (± 0.11) based on different trait definitions. The nonzero heritability indicates that susceptibility to *M. avium* ssp. *paratuberculosis* infection in Jersey cattle is influenced by genetic factors. Therefore, selection of the least susceptible animals could decrease genetic predisposition

to *M. avium* ssp. *paratuberculosis* infection in Jersey populations in future generations.

Key words: paratuberculosis, heritability, cattle, Jersey

Short Communication

Paratuberculosis, also known as Johne's disease (JD), is a chronic bacterial infection of the gastrointestinal tract in cattle, other ruminants, and many species of domestic and wild animals (Stevenson et al., 2009). *Mycobacterium avium* ssp. *paratuberculosis* is the etiological agent of JD. Paratuberculosis is contagious and *M. avium* ssp. *paratuberculosis* is transmitted to susceptible animals mostly through ingestion of bacteria from contaminated sources, such as colostrum, milk, or feces (Nielsen et al., 2008; Lombard, 2011). The National Animal Health Monitoring System Dairy 2007 study of US herds in the top 17 dairy states estimated the apparent herd-level prevalence to be at least 68% based on recovery of viable *M. avium* ssp. *paratuberculosis* in environmental fecal samples (NAHMS, 2007), whereas a recent study estimated true herd-level prevalence of *M. avium* ssp. *paratuberculosis* infection in the same herds to be 91.1% (Lombard et al., 2013). Annual losses to the dairy industry attributed to *M. avium* ssp. *paratuberculosis* infections in the United States alone have been estimated to range from \$200 million to \$1.5 billion (Stabel, 1998; Ott et al., 1999). Currently no cure exists for this infection, and vaccines, although available, postpone or reduce the onset of clinical symptoms but fail to fully protect animals from infection (Emery and Whittington, 2004).

The contribution of genetic factors to susceptibility of *M. avium* ssp. *paratuberculosis* infection in cattle has been investigated in several studies (reviewed by Kirkpatrick and Shook, 2011). The results show that susceptibility to *M. avium* ssp. *paratuberculosis* is heritable, with estimates ranging from 0.031 to 0.283 in Holsteins. The large variation in heritability estimates of *M. avium* ssp. *paratuberculosis* susceptibility stems from differences in the infection prevalence of study

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populations, sample size, and methods used in the statistical analysis, as well as differences in diagnostic tests for determination of infection status. Among the diagnostic tests used to define *M. avium* ssp. *paratuberculosis* susceptibility are serum ELISA, milk ELISA, postmortem pathology, and fecal culture (FC).

Previous heritability estimates are from studies of Holstein cattle. No report for heritability of susceptibility to *M. avium* ssp. *paratuberculosis* infection in Jersey cattle is available in the literature. Jersey is the second most common dairy breed after Holstein in the United States. Studies have shown that susceptibility to *M. avium* ssp. *paratuberculosis* may differ between breeds (Jakobsen et al., 2000; Sorge et al., 2011). Sorge et al. (2011) reported Jersey cows were 1.4 times more likely than Holsteins to test positive to milk ELISA; however, this conclusion is questionable, as breeds were confounded with herds in their data. The main objective of the current study was to estimate the heritability of susceptibility to *M. avium* ssp. *paratuberculosis* infection in the Jersey breed in the United States and, additionally, to examine variation between sires in observed and predicted frequency of test-positive daughters.

The data consisted of complete serum ELISA and partial FC results on mature cows ($n = 3,918$) from 25 commercial Jersey herds throughout the United States. These data were a subset of a larger data set (30 herds) initially collected in a cross-sectional design for the purpose of a genome-wide association study for susceptibility to *M. avium* ssp. *paratuberculosis* infection. Nomination of herds for the genome-wide association study was based on relatively high prevalence of JD evidenced by the herd's owner or veterinarian assessment of clinical signs or diagnostic testing. Blood and fecal samples were collected in one-time sampling from all mature cows (minimum age of 20 mo). All blood samples were tested by a serum ELISA (John's Testing Center ELISA; Shin et al., 2008) with 30% sensitivity and >99% specificity relative to FC (Collins et al., 2006). The ELISA optical density (OD) values for each

serum sample were converted to sample-to-positive ratios (S/P) using $(OD_{\text{Sample}} - OD_{\text{Negative control}})/(OD_{\text{Positive control}} - OD_{\text{Negative control}})$. The ELISA S/P ratios were categorized as negative (0 to 0.09), suspect (0.10 to 0.24), low positive (0.25 to 0.39), positive (0.40 to 0.99), and strong positive (≥ 1.00), as suggested by Collins (2002).

In the majority of herds, ELISA-positive and birth date-matched ELISA-negatives were selectively tested for evidence of *M. avium* ssp. *paratuberculosis* infection by FC (Collins et al., 1990). Only 2 herds had complete ELISA and FC results. The sensitivity and specificity of FC have been estimated to be approximately 74 and 100% for detection of infectious cows, respectively (Sockett et al., 1992). The selection of herds for the current study was based on criteria including whole-herd ELISA results, complete sire identification, and availability of birth and sampling dates for most cows in these herds.

The data was edited for analysis of heritability; 1 herd with <20 animals, 1 herd ($n = 49$) with a single test-positive animal lacking sire identification, and all sires with <5 daughters were removed. The edited data consisted of a total of 2,861 cows in 23 herds sired by 183 sires (Table 1). The age of cows in the data set ranged from a minimum of 20 to a maximum of 167 mo. The average and median number of daughters per sire in the edited data was 15.6 and 9, respectively. Distribution of number of daughters per sire was skewed toward many sires having few daughters; only 28 sires had >25 daughters.

A total of 4 traits (dependent variable) were defined from ELISA or FC results for analysis of genetic parameters. Parameters included (1) ELISA S/P ratios as a continuous trait; (2) ELISA OD results as a binary trait (0 = negative, 1 = positive) for which all ELISA low-positive, positive, and strong-positive cows were considered ELISA-positive (ELISA-suspect is an uncertain phenotype; therefore, it was treated as a missing value); (3) ELISA OD results as an ordered categorical trait, with categories of 1 to 5 for ELISA-negative,

Table 1. Cross-tabulation of serum ELISA by fecal culture scores for the edited data used in the heritability estimation for susceptibility to *Mycobacterium avium* ssp. *paratuberculosis* infection in Jersey cattle

Item	ELISA category					Total
	Negative ¹ (0–0.09)	Suspect (0.10–0.24)	Low positive (0.25–0.39)	Positive (0.40–0.99)	Strong positive (≥ 1.00)	
FC ² -negative	976	14	7	8	5	1,010
FC-positive	84	6	39	53	134	316
FC N/A ³	1,527	6	2	—	—	1,535
Total	2,587	26	48	61	139	2,861

¹Range of numbers in each category are sample or positive ratios.

²FC = fecal culture.

³Fecal culture result was not available.

suspect, low-positive, positive, and strong-positive, respectively; and (4) combined test as a binary trait in which animals testing positive to either ELISA (S/P ≥ 0.25) or FC were considered test-positive and cows testing negative to ELISA or to both ELISA and FC, when available, were considered test-negative. The purpose of the combined test was to use as much diagnostic information as was available and thus increase the probability of detecting infected cows. The selective FC testing and availability of complete herd FC testing for only 2 herds precluded doing heritability estimation based on FC only.

The variance components of susceptibility to *M. avium* ssp. *paratuberculosis* infection were estimated using a sire model and 3 different statistical models for various traits in which age (up to a third-order polynomial), herd, sire, and residual effects were included.

The following linear mixed model was used for the analysis of ELISA S/P ratios:

$$y_{ijk} = \mu + \beta_1(\text{age}_{ijk}) + \beta_2(\text{age}_{ijk})^2 + \text{herd}_j + \text{sire}_k + e_{ijk}, \quad [1]$$

where y_{ijk} represents the transformed ELISA S/P ratio of ijk th animal obtained by $\log_{10}(\text{S/P ratio} + 0.01)$, where a logarithmic transformation was performed to achieve an approximate normal distribution; μ is the overall mean; β_1 and β_2 are the coefficients for effects of age and age^2 , respectively; age_{ijk} is the fixed effect of age as a continuous variable calculated by the difference between ijk th animal's date of birth and date of sampling (in months); herd_j is the fixed effect of j th herd; sire_k is the random effect of the k th sire; and e_{ijk} the random residual component. Random effects were assumed to be normally distributed as

$$\text{sire} \sim N(0, \mathbf{A}\sigma_s^2), \quad [2]$$

and

$$e \sim N(0, \mathbf{I}\sigma_e^2), \quad [3]$$

where σ_s^2 represents the sire variance; σ_e^2 represents the residual variance; $\mathbf{I}$ is the identity matrix; and $\mathbf{A}$ is the sire additive numerator relationship matrix.

The ELISA binary and combined test traits were analyzed using a mixed threshold model. This model assumes that underlying disease susceptibility is a normally distributed variable known as "liability." The phenotype (*M. avium* ssp. *paratuberculosis* infection) is made discrete at a threshold k . Animals with disease liability greater than the threshold are infected, whereas

cows with liabilities less than this threshold are not infected. The threshold model used for analysis of binary traits in the current study is

$$l_{ijk} = \mu + \beta_1(\text{age}_{ijk}) + \beta_2(\text{age}_{ijk})^2 + \text{herd}_j + \text{sire}_k + e_{ijk}, \quad [4]$$

where l_{ijk} is the liability of *M. avium* ssp. *paratuberculosis* infection for animal ijk , and other terms were defined previously. The threshold was set to zero and the residual variance was set to 1. A logit link function was used.

An ordered threshold model was used to estimate underlying liability of susceptibility to *M. avium* ssp. *paratuberculosis* infection for 5 ordinal categories of ELISA S/P ratios, described previously. This model is similar to a binary threshold model, with the difference that instead of threshold k , a vector of thresholds $\mathbf{k}$ is used. The first threshold corresponding to an S/P ratio of 0.10 was set to zero, and the remaining 3 thresholds were estimated. The same independent variables as in Equation 4 were fitted. The assumptions for random effects distributions in binary and ordered threshold models were the same as in the linear model (Equations 2 and 3).

The narrow-sense heritability for susceptibility to *M. avium* ssp. *paratuberculosis* infection based on a linear model was calculated by

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2}, \quad [5]$$

where σ_s^2 represents $0.25\sigma_a^2$; σ_a^2 being additive genetic variance; and other terms as defined previously.

For a threshold model, the residual variance on the underlying scale has been estimated to be $\frac{\pi^2}{3} \sim 3.29$ for the logit link (Gilmour et al., 2009). To enable a better comparison of the estimates from linear and threshold models, the heritability of susceptibility to *M. avium* ssp. *paratuberculosis* infection from threshold models was adjusted to the underlying liability scale using the formula of Gilmour et al. (2009):

$$h_{\text{adjusted}}^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \left(\frac{\pi^2}{3}\right)}. \quad [6]$$

All analyses were executed using the restricted maximum likelihood method in ASReml software (Gilmour et al., 2009).

The observed frequency of test-positive daughters of each sire was calculated by dividing the number of test-positive daughters (based on the combined test in the edited data) by the total number of tested daughters. Additionally, for the combined test, the sire liabilities were transformed into probabilities of future *M. avium* ssp. *paratuberculosis*-infected daughters using the population average age (50.5 mo) and average herd effect. The predicted probabilities of *M. avium* ssp. *paratuberculosis* infection detection in future daughters were obtained using *predict* in ASReml (Gilmour et al., 2009). The probability of infection detection ($\hat{y}$) of each cow was estimated from the fitted model (Equation 4) using an inverse logit function.

The proportion of test-positive cows based on the combined test in the edited data varied from 1.8 to 36.3% among 23 herds, with an overall frequency of 11.8%. Intercept, age, and age squared were significantly associated with *M. avium* ssp. *paratuberculosis* infection detection for all trait definitions ($P < 0.001$).

Heritability estimates varied more than 3 fold depending on estimation methodology. The heritability estimate for ELISA S/P ratios as a continuous trait was 0.079 (SE = 0.034). The log-transformation of ELISA S/P ratios did not achieve normality. Estimated heritability of liability to *M. avium* ssp. *paratuberculosis* infection with ELISA results as a binary trait was 0.269 (0.117) and as an ordered categorical trait was 0.20 (0.106; Table 2). The heritability estimate for the combined test was 0.197 (0.093) (Table 2).

Predicted probability of future *M. avium* ssp. *paratuberculosis*-infected daughters ranged from 0.10 to 0.11 for the majority of sires (~32%). For the 28 sires with >25 daughters, the predicted probabilities ranged from 0.078 to 0.201 (average 0.118). Comparing the average observed frequency of test-positive daughters between

14 more susceptible and 14 less susceptible sires, the average frequency of test-positive daughters for the 14 more-susceptible sires was twice that for the 14 less-susceptible sires, (0.143 vs. 0.073).

The heritability estimates for *M. avium* ssp. *paratuberculosis* infection in Jersey were in the range of reported estimates for Holsteins (0.031–0.283). Using a linear model, the heritability estimate was relatively low (0.079); the estimates using binary or ordered threshold models were higher (0.197–0.269). This result is in contrast to our expectation of larger heritability estimates from linear models or ordered categorical models compared with binary threshold models. The ELISA S/P ratio as a continuous trait captures a whole spectrum of variation in susceptibility to *M. avium* ssp. *paratuberculosis* infection among animals, whereas classification of results into binary categories loses information, which is expected to result in a lower heritability estimate. As expected when using a combined ELISA and FC test, the frequency of test-positive animals increased compared with ELISA results only (11.8 vs. 8.7%). However, in contrast to previous studies (van Hulzen et al., 2011; Küpper et al., 2012) that reported higher heritability estimates for data subsets with a higher prevalence of infection, a higher prevalence (frequency of test-positive animals) in the current study did not result in a higher heritability estimate with the combined test. Nevertheless, the standard errors of these estimates were relatively large, owing to the relatively small daughter groups.

Using a linear model for transformed S/P ratios, Gonda et al. (2006) estimated the heritability of *M. avium* ssp. *paratuberculosis*-specific antibody response at 0.159 (SE = 0.09). The logarithmic transformation of ELISA S/P ratios did not achieve complete normality. This might have caused bias in the heritability

Table 2. Heritability and variance component estimates from univariate sire model analyses for susceptibility to *Mycobacterium avium* ssp. *paratuberculosis* infection in Jersey cattle

Trait	Model	h^2 (SE) ¹	Variance	
			Sire	Residual ¹
ELISA linear ²	Linear	0.079 (0.034)	0.005	0.285
ELISA binary ³	Threshold	0.269 (0.117)	0.237	3.29
ELISA ordered categorical ⁴	Ordered threshold	0.200 (0.106)	0.173	3.29
Combined test ⁵	Threshold	0.197 (0.093)	0.171	3.29

¹Heritability estimates and residual variances for (ordered) threshold models are in the underlying liability scale.

²Logarithmically transformed ELISA sample-to-positive ratios (S/P).

³The ELISA S/P ratio of 0.25 was used as a cut-off; S/P ratios ≥ 0.25 were considered ELISA positive, S/P 0 to 0.9 was considered ELISA negative, and S/P 0.1 to 0.24 was suspect and removed from analysis.

⁴Five categories of negative, suspect, low positive, positive, and strong positive were defined based on ELISA S/P ratios.

⁵Both ELISA and fecal culture results were taken into account. A cow was defined test positive if testing positive to either ELISA or fecal culture and was test negative if testing negative to ELISA or to both ELISA and fecal culture when available.

estimate (0.079) based on a linear model in the current study. For the ordered threshold model, our heritability estimate was higher than the estimate by Gonda et al. (2006) using the same model (0.20 vs. 0.091). Using a threshold model for the combined test, the heritability estimate (0.197) in the current study was almost twice the estimate of 0.102 for US Holsteins by (Gonda et al., 2006).

Predicted probability of infection detection of future daughters has been shown to vary among sires for Holsteins (Koets et al., 2000; Gonda et al., 2006; Shook et al., 2012). Predicted probability of *M. avium* ssp. *paratuberculosis* infection of daughters of the most heavily used sires ranged from <0.01 to 0.165 in Israeli Holsteins (Shook et al., 2012), 0.053 to 0.104 for US Holsteins (Gonda et al., 2006), and 0.053 to 0.088 for Dutch Holsteins (Koets et al., 2000). The range of predicted probabilities of *M. avium* ssp. *paratuberculosis* infection detection in future daughters of Jersey sires in our study was 0.078 to 0.201. The difference in predicted probabilities among these studies could reflect genetic and environmental differences between the populations or use of different diagnostic tests, statistical methods, or other criteria mentioned previously.

The ELISA and FC tests, which detect *M. avium* ssp. *paratuberculosis*-specific antibodies in the serum or viable *M. avium* ssp. *paratuberculosis* in the feces, respectively, are lowly sensitive and highly specific. This means that a test-positive animal is most likely *M. avium* ssp. *paratuberculosis*-infected, whereas a test-negative animal might be a false negative. High rates of false negatives due to low sensitivity of ELISA or FC tests could cause a negative bias in the heritability estimates. Using 2 diagnostic tests to categorize infection status of animals led to inclusion of more test-positive animals in the analysis and increased both sensitivity and frequency of test-positives. Therefore, heritability estimation with the combined test (0.197) might be closer to the true heritability of susceptibility to *M. avium* ssp. *paratuberculosis* in Jersey cattle.

Ours is the first study to report heritability estimates for susceptibility to *M. avium* ssp. *paratuberculosis* infection in Jersey cattle. The estimates of heritability were low to moderate, and ranged from 0.08 to 0.27 based on different statistical models and trait definitions. These heritability estimates are in the range of those reported previously for Holstein cattle. The nonzero heritability of *M. avium* ssp. *paratuberculosis* infection in Jersey indicates that susceptibility to *M. avium* ssp. *paratuberculosis* infection in Jersey cattle is influenced by genetic factors. Therefore, selection of less susceptible animals could reduce genetic predisposition to *M. avium* ssp. *paratuberculosis* infection in Jersey cattle in future generations.

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