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Genetic Variation of *Mycobacterium avium* ssp. *paratuberculosis* Infection in US Holsteins

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ABSTRACT

The objective of this study was to estimate genetic variability of *Mycobacterium avium* ssp. *paratuberculosis* infection in US Holsteins. Blood and fecal samples were collected primarily from daughters of 12 bulls in their second or third lactation. Routine disease testing of the sires documented that they were not infected. Herds without a "suspect" or positive ELISA (sample/positive ratio ≥ 0.10) or positive fecal culture test were deleted from the data set. The remaining 4,603 cows from 238 herds and 46 sires were used to estimate heritability of *M. paratuberculosis* infection. Heritability was estimated with 3 Johne's disease diagnostic tests: 1) fecal culture alone, 2) serum antibody ELISA alone, and 3) both tests (combined) with a positive animal defined as all animals with either a positive fecal culture or ELISA test. Four statistical models were used to estimate heritability: 1) linear (ELISA), 2) threshold (fecal culture and combined), 3) ordered threshold (ELISA), and 4) bivariate linear-threshold (ELISA-fecal culture). A sire model and Bayesian approach using Markov chain Monte Carlo methods were used in each case. Heritability of infection based on the fecal culture test was 0.153 [posterior standard deviation (PSD) = 0.115]. Heritability with the ELISA was 0.159 (PSD = 0.090) with a linear model and 0.091 (PSD = 0.053) with an ordered threshold model. Heritability of the combined tests was 0.102 (PSD = 0.066). Heritability estimates of fecal culture and ELISA with the bivariate model varied slightly from estimates obtained with the univariate models (0.125 and 0.183, respectively), with a corresponding increase in precision (PSD = 0.096 and 0.082, respectively). This study demonstrates that exploitable genetic variation exists in dairy cattle for *M. paratuberculosis* infection susceptibility.

Key words: Johne's disease, genetic variation, heritability, Holstein

INTRODUCTION

Johne's disease is an enteric infection caused by *Mycobacterium paratuberculosis*, also called *Mycobacterium avium* ssp. *paratuberculosis*. The disease is found in numerous species including cattle (Collins, 2003). Cattle normally become infected with *M. paratuberculosis* as calves, but clinical signs of infection usually do not appear until the cow's second or third lactation (Jubb and Galvin, 2000). Clinical signs of infection include weight loss, diarrhea, decreased milk production, and eventually death. No treatment for the disease exists and controlling the disease is difficult due to its long latent period. Johne's disease is a worldwide problem; almost all countries testing for the disease have found *M. paratuberculosis*-infected animals. One study reported an apparent herd-level prevalence of 21.6% across all herd sizes in the US, with 40% of herds with >300 cows having the disease (NAHMS, 1997). Johne's disease costs the US dairy industry $200 to $250 million annually (Ott et al., 1999).

Genetic variability of susceptibility to bacterial infections has been estimated for some cattle diseases. Clinical mastitis has been the most extensively studied, with heritability estimates ranging from 0.01 to 0.09 (Carlen et al., 2004; Heringstad et al., 2004; Odegard et al., 2004). Heritability estimates for metritis have also been reported (ranging from 0 to 0.26; Lin et al., 1989; Simerl et al., 1991; Zwald et al., 2004).

Two studies have estimated heritability of Johne's disease susceptibility. In the first study, based on postmortem carcass evaluations, heritability ranged from <0.01 to 0.09 in 3,020 Dutch dairy cattle, according to a threshold model (Koets et al., 2000). Although postmortem carcass evaluations are likely the most accurate method of identifying *M. paratuberculosis*-infected animals, this method is not practical for routine disease diagnosis. Numerous diagnostic tests for Johne's disease have been developed including serum and milk ELISA, fecal bacterial culture, skin tests, and IFN-γ assays (Collins and Manning, 2005). The only diagnostic test used to estimate heritability of Johne's disease susceptibility has been the milk ELISA (Mortensen et

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al., 2004). In this second study of *M. paratuberculosis* infection susceptibility, the estimated heritability was 0.102 in 11,535 Danish Holsteins with a bivariate linear model (the other dependent variable being daily milk yield).

The heritability of Johne's disease susceptibility may vary with the diagnostic test. For example, SCS is often used as a proxy for mastitis; however, heritability estimates were larger for SCS (0.10 to 0.14) than for clinical mastitis as measured by veterinary treatments (0.01 to 0.03) with a bivariate linear model (Carlen et al., 2004; Odegard et al., 2004). The statistical model may also influence heritability estimates; heritability of clinical mastitis was larger when estimated with a threshold model (0.05 to 0.09; Heringstad et al., 2004).

The objective of this study was to estimate heritability of *M. paratuberculosis* infection as measured by serum ELISA and fecal culture of *M. paratuberculosis* in US Holsteins. Johne's disease is a good candidate for genetic selection because it is incurable and an effective vaccine is not available.

MATERIALS AND METHODS

Data Collection

Daughters of 12 Holstein sires were tested for Johne's disease as part of a daughter design to detect disease resistance loci (not part of this study). Two criteria were used to select sires: 1) large number of daughters in production at time of sampling, and 2) low genetic relationships among sires. Of 66 possible pairings of sires, relationships were 0.25 for 1 pair of sires, 0.125 for 7 pairs, 0.0625 for 13 pairs, and lower for the remaining 45 pairs. Routine, frequent testing of these bulls for Johne's disease documented that all bulls were disease free (as reported by owners of the bulls). In addition to these sires, a smaller number of daughters from 214 other sires from a preliminary study were included in the data set. These daughters were from 28 herds that tested all cows for Johne's disease. Pedigree records were obtained from the Animal Improvement Programs Laboratory, USDA (Beltsville, MD).

Serum and fecal samples were collected from daughters of these sires in US dairy herds by the respective herd veterinarians. Herds were selected on the following criteria: 1) participation in the US Dairy Herd Improvement program, and 2) presence of at least 5 cows in second or third lactation sired by one or more project bulls. Cows in second or third lactation are more likely to produce antibodies to *M. paratuberculosis* than are cows in first lactation (Jubb and Galvin, 2000). Prevalence of infection in later lactations (>3) would be biased downwards because some infected cows would already have been culled from the herd. Approximately 67% of cows in this study were in second or third lactation; the rest were primarily first-lactation cows. None of the sampled herds was vaccinated for Johne's disease. Approximately 32% of samples were obtained from herds in Wisconsin, 16% from herds in California, and the remaining samples from herds scattered throughout the United States. Data were collected from July 1999 to July 2003, although 66% of samples were collected in 2001 and 2002. Samples from the preliminary study were collected in 1999 and 2000. A total of 5,611 cows from 300 herds were sampled.

Disease Diagnosis

An antibody test on the serum samples was performed using a USDA-licensed Johne's ELISA kit (Idexx Laboratories, Inc., Westbrook, ME). The ELISA measures the amount of *M. paratuberculosis* antibody present in the cow's serum, which is an indicator of *M. paratuberculosis* infection and, therefore, susceptibility to infection. Optical density values for the serum, positive control, and negative control were converted to sample-to-positive (**S/P**) ratios. Fecal culture of *M. paratuberculosis* was performed using the radiometric (Bactec) method (Collins et al., 1990). Diagnostic tests were performed by the Johne's Testing Center at the School of Veterinary Medicine, University of Wisconsin-Madison.

Infection by *M. paratuberculosis* was defined in 3 ways: 1) fecal culture alone, 2) ELISA alone, or 3) combined fecal culture and ELISA. Cows with *M. paratuberculosis* cultured from feces after incubation for 12 wk were considered fecal culture-positive. For the ELISA, transformed S/P ratios were either used directly as the phenotype or S/P ratios were placed into ordered categories as follows: 1) negative (S/P ratio 0 to 0.09), 2) suspect (0.10 to 0.24), 3) weak positive (0.25 to 0.39), 4) positive (0.40 to 0.99), and 5) strong positive (≥1.00). These categories were based on recommendations by the Johne's Testing Center at the University of Wisconsin-Madison (Collins, 2002). For the combined test, ELISA and fecal culture were used in parallel; that is, cows with either a positive fecal culture or ELISA S/P ratio ≥0.25 were diagnosed as *M. paratuberculosis*-infected.

Data Editing

Herds without a suspect or test-positive cow were removed from the data set because there was no evidence of exposure to *M. paratuberculosis* in our data. Our case definition for this purpose was defined as either a positive fecal culture or ELISA S/P ratio ≥0.10. A lower S/P ratio was used because only a small number

Table 1. Percentage of cows positive for *Mycobacterium paratuberculosis* by serum, fecal, and combined tests in complete and edited datasets

	Number of cows		
	Positive	Total	% Positive
Complete data set			
ELISA[1]	364	5,608	6.49
Fecal culture	149	4,545	3.28
Combined tests[2]	425	5,611	7.57
Edited data set[3]			
ELISA[1]	313	4,603	6.80
Fecal culture	117	3,694	3.17
Combined tests[2]	359	4,603	7.80

[1]A sample/positive (S/P) ratio ≥ 0.25 was scored as a positive ELISA.

[2]Combining ELISA and fecal culture test results, a *M. paratuberculosis*-infected cow was a cow with an ELISA S/P ≥ 0.25 or positive fecal culture.

[3]The edited data set removed herds without a suspect or positive test for *M. paratuberculosis* (defined as fecal culture-positive or ELISA S/P ≥ 0.10) and half-sib families with less than 5 daughters.

of cows from most herds were tested for the disease. A higher S/P ratio threshold would have removed more herds from the study, decreasing precision of our estimates further and potentially removing many cows that were exposed to *M. paratuberculosis* but not susceptible to the infection. After removing 62 herds without suspect or test-positive cows, half-sib families with fewer than 5 cows were removed because these families provided little information for estimating heritability. Fourth- and fifth-parity cows were grouped with third-parity cows because our data set contained very few of these animals. This edited data set consisted of records from 4,603 cows from 46 sires in 238 herds. Of these cows, 4,233 (92.0%) were daughters of the 12 project sires. All 12 project half-sib families remained in the edited data set. Numbers of cows in lactations 1, 2, and 3 were 1,525 (33.13%), 2,239 (48.64%), and 839 (18.23%), respectively. Apparent *M. paratuberculosis* infection prevalences in the edited and complete datasets were similar (Table 1). One-third of fecal culture-positive cows were ELISA-negative (S/P < 0.10; Table 2). About 23.3 and 5% of fecal culture-negative cows were ELISA-positive (S/P ≥ 0.10 and ≥ 0.25, respectively). This data set was used to estimate heritability with the ELISA and combined test. Fecal cultures were either not performed or the culture was contaminated with microbes other than *M. paratuberculosis* for 909 cows. Therefore, 3,694 cows from 45 sires in 226 herds were used to estimate heritability with the fecal culture.

Statistical Models

Parameters were estimated using 5 mixed models: 1) a linear model with the ELISA, 2) a threshold model with fecal culture, 3) a threshold model with the combined tests, 4) an ordered threshold model with the ELISA, and 5) a bivariate linear (ELISA)-threshold (fecal culture) model. A Bayesian approach using Markov chain Monte Carlo methods was used for all models (Sorensen et al., 1995; Sorensen and Gianola, 2002). A flat prior based on a previous heritability estimate of infection susceptibility (0.102) was used (Mortensen et al., 2004). The prior was selected based on this heritability estimate as opposed to an estimate based on postmortem carcass evaluations (Koets et al., 2000) because the diagnostic test (milk ELISA) was more similar to the tests conducted in the present study. Parameters were estimated with a sire model, with relationships between sires traced back 3 generations. In a preliminary study, DIM was included as a covariate in all models in addition to parity and herd, but was not significantly associated with *M. paratuberculosis* infection.

Linear Model. A mixed linear model was used to analyze log transformed ELISA S/P ratios as follows:

$$ELISA_T = \log_{10}(ELISA + 0.01)$$

where $ELISA_T$ = transformed ELISA S/P ratio. The S/P ratios were transformed to attain a more nearly normal frequency distribution. The fitted model was

$$y_{ijkl} = p_i + s_j + h_k + e_{ijkl}$$

Table 2. Cross-tabulation of serum ELISA score by fecal score for the edited data set

	ELISA category[1]					
	Negative (0 to 0.09)	Suspect (0.10 to 0.24)	Weak positive (0.25 to 0.39)	Positive (0.40 to 0.99)	Strong positive (≥ 1.00)	Total
Fecal culture-negative	2,744	654	75	65	39	3,577
Fecal culture-positive	39	7	7	22	42	117
Fecal N/A[2]	681	165	24	22	17	909
Total	3,464	826	106	109	98	4,603

[1]Range of numbers in each category are sample/positive ratios.

[2]Fecal culture contaminated by growth of another organism or was not available for testing.

where y_{ijkl} = transformed ELISA S/P ratio, p_i = fixed effect of parity, s_j = random sire effect, h_k = random herd effect, and e_{ijkl} = residual. Random effects for the univariate models were assumed to be normally distributed as follows:

$$\mathbf{h} \sim N(0, \mathbf{I}\sigma_h^2) \text{ and } \mathbf{s} \sim N(0, \mathbf{A}\sigma_s^2)$$

where $\mathbf{h}$ = herd effect, $\mathbf{s}$ = sire effect, $\mathbf{I}$ = identity matrix, σ_h^2 = herd variance, $\mathbf{A}$ = sire additive numerator relationship matrix, and σ_s^2 = sire variance. Residuals were assumed to be normally distributed with mean zero and variance σ_e^2, where σ_e^2 = residual variance. Parameters were estimated using original Linear.F90 software developed by Y. M. Chang (provided upon request).

Threshold Model. A mixed threshold model was used to estimate parameters with the fecal culture and combined tests. This model assumes an underlying normally distributed disease liability. Cows with liability to disease greater than the threshold (zero) were infected, and cows with liability to disease less than the threshold were noninfected. A cumulative normal density function was used to link disease liability with probability of infection (either 0 or 1). The threshold model was

$$l_{ijkl} = p_i + s_j + h_k + e_{ijkl}$$

where l_{ijkl} = liability for cow $ijkl$, and other terms were defined previously. The threshold was set to zero and residual variance was set to 1.00.

For the combined test, sire families were evaluated based on their predicted probability of *M. paratuberculosis*-infected daughters. Probabilities were estimated from the posterior mean of the sire PTA for liability as follows (Chang et al., 2004):

$$\Pr(y_i = 1|\mu,\bar{s}_i \approx 1 - \Phi(\mu + \bar{s}_i)$$

where y_{ij} = binary response variable with value 0 if cow is noninfected and 1 if infected, μ = population mean liability, $\bar{s}_i$ = posterior mean PTA of liability for sire i, and $\Phi(.)$ = cumulative normal density function. The sire liabilities were transformed into probabilities of future *M. paratuberculosis*-infected daughters relative to the overall population mean. A 95% confidence interval for this probability was calculated for each sire as $\Phi(\bar{s}_i \pm$ 1.96 posterior standard deviation, **PSD**). Sire PTA probabilities are a nonlinear transformation of sire PTA liabilities. As a result, sires were ranked in the same order on both scales but the probability is more easily interpretable. Parameters and probabilities were estimated using Probit.F90 software developed by Y. M. Chang (Chang et al., 2004; available upon request).

Ordered Threshold Model. Cows were placed into ordered categories based on their ELISA S/P ratios, as described previously. The ordered threshold model estimated underlying normally distributed liability of *M. paratuberculosis* infection. The same independent variables were used as in the linear model:

$$\lambda_{ijkl} = p_i + s_j + h_k + e_{ijkl}$$

where λ_{ijkl} = liability of *M. paratuberculosis* infection. Four thresholds were estimated with this model, with the first threshold, corresponding to an S/P ratio of 0.10, set to zero. For example, if a cow is in category c, its liability of *M. paratuberculosis* infection falls between thresholds T_{c-1} and T_c. Parameters were estimated using original Ordinal.F90 software developed by Y. M. Chang (available upon request).

Bivariate Threshold-Linear Model. A bivariate model with fecal culture and ELISA results was used to estimate heritability of *M. paratuberculosis* infection as follows:

$$\begin{bmatrix}\mathbf{Y_1}\\ \mathbf{Y_2}\end{bmatrix} = \begin{bmatrix}\mathbf{X} & \mathbf{0}\\ \mathbf{0} & \mathbf{X}\end{bmatrix}\begin{bmatrix}\beta_1\\ \beta_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z_h} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_h}\end{bmatrix}\begin{bmatrix}\mathbf{h_1}\\ \mathbf{h_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z_s} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_s}\end{bmatrix}\begin{bmatrix}\mathbf{s_1}\\ \mathbf{s_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z_e} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z_e}\end{bmatrix}\begin{bmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix}$$

where $\mathbf{Y_1}$ = transformed ELISA S/P ratios as described for the linear model, $\mathbf{Y_2}$ = liability for fecal culture test, $\mathbf{X}$ = incidence matrix relating fixed effect of parity β to observations, $\mathbf{Z_h}$ = incidence matrix relating random herd effects $\mathbf{h}$ to observations, $\mathbf{Z_s}$ = incidence matrix relating random sire effects s to observations, and $\mathbf{Z_e}$ = incidence matrix relating residuals e to observations. Herd and sire effects were assumed normally distributed as follows:

$$\begin{pmatrix}\mathbf{h_1}\\ \mathbf{h_2}\end{pmatrix} \sim N(0, H \otimes \mathbf{I}) \begin{pmatrix}\mathbf{s_1}\\ \mathbf{s_2}\end{pmatrix} \sim N(0, G \otimes \mathbf{A})$$

$$\begin{pmatrix}\mathbf{e_1}\\ \mathbf{e_2}\end{pmatrix} \sim N(0, R \otimes \mathbf{I})$$

where $\mathbf{h_i}$ = herd effect for the ELISA or fecal culture phenotypes, $\mathbf{s_i}$ = sire effect for the ELISA or fecal culture phenotypes, $\mathbf{H}$ = herd variance-covariance matrix, $\mathbf{G}$ = sire variance-covariance matrix, $\mathbf{R}$ = residual variance-covariance matrix, $\mathbf{I}$ = identity matrix, and $\mathbf{A}$ = sire additive numerator relationship matrix.

As for the threshold model, a cumulative normal density function was used to link fecal culture liability with

Table 3. Apparent prevalence of *Mycobacterium paratuberculosis* infection in 12 largest half-sib families

Half-sib family[1]	No. of daughters	Unadjusted apparent prevalence of infection[2]	Predicted apparent prevalence of future daughters[3]	Lower 95% boundary[4]	Upper 95% boundary[4]
A	252	0.087	0.080	0.056	0.121
B	338	0.059	0.069	0.047	0.108
C	113	0.124	0.104	0.068	0.155
D	132	0.030	0.053	0.027	0.094
E	138	0.123	0.101	0.063	0.150
F	496	0.069	0.074	0.049	0.101
G	431	0.067	0.076	0.048	0.111
H	620	0.074	0.083	0.060	0.108
I	424	0.080	0.089	0.063	0.123
J	570	0.068	0.072	0.048	0.099
K	319	0.047	0.060	0.040	0.094
L	400	0.098	0.090	0.066	0.133
Average	353	0.077	0.079		

[1]Letters are used to identify sire families to protect confidentiality of bulls.

[2]ELISA (sample/positive ≥ 0.25) or fecal culture-positive cows were classified as *M. paratuberculosis*-infected.

[3]Based on combined threshold model.

[4]Confidence interval calculated as Φ(sire PTA for liability ± 1.96 PSD).

probability of *M. paratuberculosis* infection (either 0 or 1). The bivariate model also estimates the genetic, herd, and residual correlations between these traits. Parameters were estimated using original Binary_Linear.F90 software developed by Y. M. Chang (available upon request).

Heritability Estimation. Within-herd heritabilities were calculated for each Monte Carlo Markov chain iteration:

$$h^2 = \frac{4 \times \sigma_{\mathbf{s}}^2}{\sigma_{\mathbf{s}}^2 + \sigma_{\mathbf{e}}^2}$$

where σ_s^2 and σ_e^2 are the sire and residual variances, respectively. Posterior means and standard deviations are reported.

RESULTS

Prevalence of infection as measured by the combined test varied among the 12 largest half-sib families (Table 3). Number of daughters per sire family ranged from 113 to 620. Overall frequency of *M. paratuberculosis* infection for these sire families, based on the combined test, was 0.077. Predicted probability of *M. paratuberculosis*-infected daughters ranged from 0.053 (n = 132) for sire family D to 0.104 for sire family C (n = 113), with a mean of 0.079. Among these 12 sires, daughter prevalence for the highest sire was twice that for the lowest sire. A similar range was observed with all 46 sires (Figure 1). The predicted probability of *M. paratuberculosis*-infected daughters adjusts for differences among sires due to lactation number and herd prevalence. Therefore, predicted probabilities account for partial confounding between sires and herds. The predicted probability is also regressed toward the mean on the basis of number of daughters.

Predicted probabilities of *M. paratuberculosis* infection were 0.065 for parity 1, 0.059 for parity 2, and 0.092 for parity ≥ 3. Previous studies have shown an increase in probability of detecting *M. paratuberculosis* infection among older cows (Jakobsen et al., 2000; Niel-

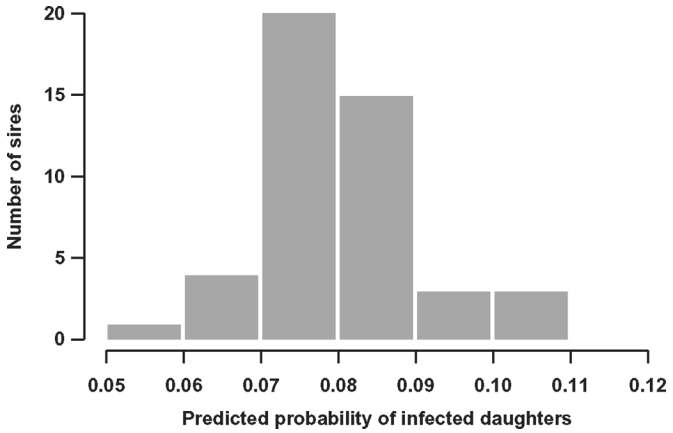

Figure 1. Distribution of predicted probabilities of *Mycobacterium avium* ssp. *paratuberculosis*-infected daughters for all 46 sires. Probabilities were estimated for the combined phenotype.

Table 4. Estimated variances and heritability with their posterior standard deviations (PSD) for *Mycobacterium paratuberculosis* infection based on diagnostic test and statistical model[1]

Diagnostic test	Model	Variance (PSD)			Heritability[2] (PSD)
		Sire	Herd	Residual	
Fecal culture	Threshold	0.041 (0.033)	0.352 (0.119)	1.0[6]	0.153 (0.115)
	Bivariate threshold[3]	0.039 (0.033)	0.245 (0.092)	1.0[6]	0.125 (0.096)
ELISA	Linear	0.017 (0.010)	0.053 (0.008)	0.401 (0.009)	0.159 (0.090)
	Bivariate linear[3]	0.012 (0.007)	0.052 (0.008)	0.401 (0.009)	0.183 (0.082)
	Ordered threshold[4]	0.023 (0.014)	0.125 (0.024)	1.0[6]	0.091 (0.053)
Combined[5]	Threshold	0.027 (0.018)	0.131 (0.039)	1.0[6]	0.102 (0.066)

[1]Sire models were used throughout.

[2]Within-herd heritabilities estimated (herd variance not included in denominator).

[3]Dependent variables in the bivariate model were the ELISA and fecal culture.

[4]Cows were placed into ordered categories based on their ELISA. Categories were the following: negative [sample/positive (S/P) ≤ 0.09], suspect (0.10 ≤ S/P ≤ 0.24), weak positive (0.25 ≤ S/P ≤ 0.39), positive (0.40 ≤ S/P ≤ 0.99), and strong positive (S/P ≥ 1.00) (Collins, 2002).

[5]Cows were diagnosed as disease positive if either their ELISA (S/P ≥ 0.25) or fecal culture test was positive. Otherwise, cows were diagnosed as noninfected if both tests were negative.

[6]Residual variances were set to 1 for threshold model.

sen et al., 2002). Our estimate for third-parity cows was perhaps slightly inflated because this parity also included fourth- and fifth-parity cows; however, cows with parity > 3 represented only about 5% of third-parity cows (less than 1% of all cows). Removing fourth- and fifth-parity cows from the data set did not change our heritability estimates.

Heritability estimates were similar between diagnostic tests (Table 4). For each model, the frequency distribution of posterior sire variance estimates was skewed to the left; however, using the median did not significantly change the heritability estimate. Estimated heritability of liability to *M. paratuberculosis* infection with the fecal culture and combined tests was 0.153 and 0.102, respectively, with a threshold model. Although a slightly larger heritability was obtained with the fecal culture test, the PSD values were both rather large (Table 4).

Two models were used to estimate heritability of the ELISA. In the first model, the S/P ratios were transformed to approximate normality and heritability was estimated using a linear model. Heritability of the ELISA using a linear model was 0.159. In the second model, cows were placed into ordered categories based on their ELISA S/P ratios. Estimated heritability of liability to the ELISA using this model was 0.091. This estimate was smaller than the estimate obtained with the linear model (Table 4). The PSD of heritability with the ordered threshold model was 40% less than for the linear model.

Heritability of the ELISA and fecal culture was estimated using a bivariate model, which accounted for the correlation between these tests. For the ELISA, the log-transformed S/P ratios were used. With the bivariate model, the ELISA estimate of heritability was 0.183 and the fecal culture estimate was 0.125. The ELISA and fecal culture estimates were slightly larger and smaller, respectively, than their univariate estimates. The PSD were also smaller with the bivariate model relative to their PSD in the univariate models, indicating a small increase in precision. The estimated genetic correlation between ELISA and fecal culture was –0.211 (PSD = 0.356). Given the large coefficient of variation (1.687), no conclusions can be drawn from this estimate. Estimated herd and residual correlations were 0.238 (PSD = 0.144) and 0.491 (PSD = 0.029), respectively.

The coefficient of variation of the estimates was larger for the combined test than the ELISA, indicating that the heritability estimate for the ELISA was more precise. However, the combined test also used information from both diagnostic tests (fecal culture and ELISA) for disease diagnosis. As a result, the combined test has a higher diagnostic sensitivity than either test alone (sensitivity is the frequency of observing a positive test result when the cow is *M. paratuberculosis*-infected; Collins and Sockett, 1993). Therefore, estimated heritability for infection based on the combined test (0.102) may be closest to true heritability of *M. paratuberculosis* infection.

DISCUSSION

Several factors decreased the precision of our heritability estimates. Diagnostic tests were collected in commercial herds for a project to map major loci affecting Johne's disease susceptibility (not part of this article). The design of this study in terms of family size and

number of families was chosen to optimize detection of major loci affecting *M. paratuberculosis* infection susceptibility. For this purpose, large family sizes (and consequently, small numbers of families) and minimal genetic relationships among selected sire families were necessary. A larger number of smaller more related families would have been more effective for estimating genetic variances and heritabilities. The false-positive rate using ELISA and fecal culture tests in parallel is 0.005 (Collins and Sockett, 1993). Unfortunately, diagnostic tests for *M. paratuberculosis* infection have high false-negative rates; with the combined test, the false-negative rate is 0.31 when S/P ratios ≥ 0.25 are taken to indicate *M. paratuberculosis* infection (Collins and Sockett, 1993). Testing cows multiple times during their lactations would have increased sensitivity. In comparison, Mortensen et al., (2004) tested cows 1 to 3 times for infection, but used only one diagnostic test (milk ELISA).

An ELISA S/P ratio cut-off of 0.25 was used to classify cows as *M. paratuberculosis*-infected with the combined test. A larger S/P ratio cut-off would have decreased the number of false-positives, but increased the number of false-negative results. For purposes of genetic analysis, using a diagnostic test that more precisely discriminates between positive and negative animals is more important than setting a high threshold to avoid the cost of a false-positive result to producers. By using a lower cut-off value, the number of false-positives would be increased, but the number of false-negatives would be decreased. The false-negative rates of fecal culture and ELISA are higher than the false-positive rates, so false-negatives were more of a concern in this study. Heritability of the combined test was estimated with ELISA cut-offs of 0.10 and 0.40, and these estimates were similar to our reported estimate (data not shown).

A previous study estimated sires' relative risk of having *M. paratuberculosis*-infected daughters (Koets et al., 2000). In that study, relative risk for the highest 2.5% of sires (among 1,761) was 1.16 times the risk for the lowest 2.5% of sires. Based on the mean (μ) and standard deviation (SD) of all 46 sire PTA probabilities in the present study and assuming a normal distribution, the 95% confidence interval for the probabilities was calculated as ($\pm$1.96 SD) + μ. In the present study, predicted probability of *M. paratuberculosis*-infected daughters for the highest 2.5% of sires (among 46) was 1.73 times the risk for the lowest 2.5% of sires. The difference between outcomes might be due to the number of daughters per bull. Koets et al. (2000) had 3,020 cows sired by 1,761 bulls; <2 daughters per bull on average. Their sire evaluations would be very strongly regressed to the population average due to the small number of daughters per sire. In contrast, our study had an average of 100 daughters per sire, so the regression of breeding value on phenotype was on average much larger.

Our heritability of the combined test is the most similar to estimates obtained in previous studies of *M. paratuberculosis* infection. Heritability of *M. paratuberculosis* infection measured with a milk ELISA was 0.102 in a previous study with Danish Holsteins (Mortensen et al., 2004). In their study, heritability was estimated with a bivariate linear model with dependent variables milk ELISA and test-day milk yield. This estimate is comparable with our estimate with the combined test (also 0.102). Heritability of *M. paratuberculosis* infection measured by postmortem carcass evaluations was 0.06 with vaccinated and nonvaccinated animals (n = 3,020) but less than 0.01 for nonvaccinated cows alone (n = 760; Koets et al., 2000). The nonvaccinated subset likely did not have enough data for an accurate heritability estimate (the standard error was not reported for the nonvaccinated subset). Therefore, the estimate including vaccinated and nonvaccinated animals is likely the most reliable. Our heritability estimate with the combined test (0.102), using only nonvaccinated cows, was similar to this more reliable estimate (0.06) by Koets et al., (2000).

Transmission of *M. paratuberculosis* infection through semen is plausible given the extensive distribution of *M. paratuberculosis* in accessory sex glands of breeding bulls (Ayele et al., 2004). Sexual transmission of the infection could also cause differences between prevalences of *M. paratuberculosis* infections in our half-sib families and bias our heritability estimates. The 12 bulls with the largest number of daughters were tested routinely for Johne's disease and did not test positive for *M. paratuberculosis* infection (personal communication from bull owners). However, sensitivity of diagnostic tests for Johne's disease is low. Therefore, a small possibility exists that bulls that tested negative for *M. paratuberculosis* infection were false-negatives and *M. paratuberculosis*-infected.

Our heritability estimates were similar regardless of the diagnostic test used to define a cow as infected. This result is not surprising because each test measures *M. paratuberculosis* infection (Gardner et al., 2000). Nevertheless, ELISA and fecal culture could be measuring genetically different responses to infection. Some *M. paratuberculosis*-infected cows shed the bacteria in feces but do not produce serum antibodies to *M. paratuberculosis* and vice versa. Some genes that affect serum antibody production to *M. paratuberculosis* differ from genes that affect *M. paratuberculosis* shedding in feces. The small difference between fecal culture and ELISA heritability estimates could be caused by the lack of complete pleiotropy between genes affecting each test

result. However, differences in heritability between diagnostic tests could also be the result of sampling variation and the different statistical models used to estimate variance components. Practically, veterinarians would diagnose disease status using the combined test when both the fecal culture and ELISA are available. Often, only one test is available, so heritability for each test alone was estimated.

The ordered threshold model estimated a smaller heritability with the ELISA than did the linear model. The ELISA S/P ratios were transformed to approximate normality for the linear model. Nevertheless, the transformation did not achieve complete normality. The ordered threshold does not require a normally distributed phenotype. Although some information is lost because ELISA S/P ratios are grouped into categories, we believe the ordered threshold model is most appropriate for estimating heritability of the ELISA.

Heritability estimates for fecal culture and ELISA with the bivariate model differed slightly from estimates with the univariate models. Despite both tests measuring *M. paratuberculosis* infection status, a negative genetic correlation (–0.211) between these tests was found. However, precision of the genetic correlation estimate in this study was very low (PSD = 0.356; CV = 1.687), for reasons similar to the lack of precision of our heritability estimates. Conclusions cannot be drawn from our genetic correlation estimate because of its low precision.

Infection with *M. paratuberculosis* is determined by host and bacterial genetic and environmental factors. One important environmental factor is the degree of exposure to *M. paratuberculosis*. Cows with higher levels of *M. paratuberculosis* exposure are more likely to become infected with the organism. An additional source of variation may be an interaction between host genetics and exposure level. For example, certain genes may only affect *M. paratuberculosis* infection when exposure to the organism is high. Unfortunately, cows were not uniformly exposed to *M. paratuberculosis* and exposure cannot reliably be measured under field conditions. Herds without cows having either a positive fecal culture or ELISA S/P ratio ≥ 0.10 were not included in this study. This criterion should have removed from the data set cows that were not exposed to *M. paratuberculosis* and thereby increased the likelihood of at least some level of exposure for all cows in this study.

CONCLUSIONS

Predicted prevalence of *M. paratuberculosis* infection varied among our 12 largest half-sib families, ranging from 0.053 to 0.104 (n range = 113 to 620). The heritability estimate of 0.102 for the combined test is our most credible estimate because it is based on a more comprehensive phenotype (combination of the fecal culture and ELISA results). This study confirmed previous heritability estimates of *M. paratuberculosis* infection. Data from this and other studies show that the ELISA antibody levels, whether used with serum or milk, and fecal culture results are similarly heritable. However, heritability estimates for *M. paratuberculosis* infection are imprecise because of the binary nature of most Johne's disease tests, low diagnostic sensitivity of these tests, and cost of collecting sufficient diagnostic test phenotypes for accurate parameter estimation. This study is the first to estimate heritability of *M. paratuberculosis* infection with the fecal culture test and serum ELISA. Despite a low heritability, this study and others demonstrate that susceptibility to *M. paratuberculosis* infection is heritable and that selecting for increased Johne's disease resistance in cattle should be possible.

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