Genetics of tuberculosis in Irish Holstein-Friesian dairy herds

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ABSTRACT

Information is lacking on genetic parameters for tuberculosis (TB) susceptibility in dairy cattle. *Mycobacterium bovis* is the principal agent of tuberculosis in cattle. The objective of this study was to quantify the genetic variation present among Irish Holstein-Friesian dairy herds in their susceptibility to *M. bovis* infection. A total of 15,182 cow and 8,104 heifer single intradermal comparative tuberculin test (SICTT, a test for *M. bovis* exposure and presumed infection) records from November 1, 2002, to October 31, 2005, were available for inclusion in the analysis. Data on observed carcass TB lesions from abattoirs were also available for inclusion in the analysis. The only animals retained were those present in a herd during episodes in which at least 2 animals showed evidence of infection; this ensured a high likelihood of exposure to *M. bovis*. Linear animal models, and sire and animal threshold models were used to estimate the variance components for susceptibility to *M. bovis*-purified protein derivative (PPD) responsiveness and confirmed *M. bovis* infection. The heritability estimates from the threshold sire models were biased upward because the relatedness between dam-daughter pairs was ignored. The threshold animal model produced heritability estimates of 0.14 in cows and 0.12 in heifers for susceptibility to *M. bovis*-PPD responsiveness, and 0.18 in cows for confirmed *M. bovis* infection susceptibility. Therefore, exploitable genetic variation exists among Irish dairy cows for susceptibility to *M. bovis* infection. Sire rankings from the linear and threshold animal models were similar, indicating that either model could be used for the analysis of susceptibility to *M. bovis*-PPD responsiveness. A favorable genetic correlation close to unity was observed between susceptibility to confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness, indicating that direct se-

lection for resistance to *M. bovis*-PPD responsiveness will indirectly reduce susceptibility to confirmed *M. bovis* infection. Data from the national TB eradication program could be used routinely to estimate breeding values for susceptibility to *M. bovis* infection.

Key words: *Mycobacterium bovis*, tuberculosis, genetics, heritability

INTRODUCTION

Tuberculosis (TB) is a chronic bacterial disease in humans and animals characterized by the progressive development of granulomatous lesions or tubercles in diseased tissues (Thoen and Bloom, 1995). The majority of human TB cases result from infection with *Mycobacterium tuberculosis*; however, a small proportion are caused by *Mycobacterium bovis* from cattle and other domesticated mammals (O'Reilly and Daborn, 1995). To reduce the zoonotic risk, most developed countries have attempted to eradicate TB from their domestic animals. *Mycobacterium bovis* infection in cattle is generally chronic, and animals may remain subclinical for long periods before presenting with clinical signs. Further, cattle may become infectious long before they exhibit clinical signs or lesions. Consequently, the mainstay of TB control in cattle lies in the early detection and removal of *M. bovis*-infected animals. In countries with an ongoing eradication program, clinical cases of *M. bovis* infection are rare.

The cell-mediated immune response is the prominent immunological reaction in *M. bovis*-infected cattle. The tuberculin test, which measures the cell-mediated immune response to *M. bovis*, is the international standard for antemortem diagnosis of bovine TB in cattle (De la Rua-Domenech et al., 2006). In Ireland, *Mycobacterium avium* and other *Mycobacterium* spp. are prevalent in the environment, causing nonspecific sensitization to the tuberculin of *M. bovis*. Thus, the single intradermal comparative tuberculin test (SICTT) is used, as opposed to the single intradermal test (More and Good, 2006). The SICTT involves concurrent intradermal

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injection of *M. bovis*-purified protein derivative (PPD) and *M. avium*-PPD antigens, and works on the premise that *M. bovis*-infected cattle tend to show a greater response to *M. bovis*-PPD than to *M. avium*-PPD, whereas infection with other *Mycobacterium* spp. promotes the inverse (De la Rua-Domenech et al., 2006).

In 1954, Ireland introduced a TB eradication policy to reduce the estimated 17% animal incidence of bovine TB [Department of Agriculture, Fisheries and Food (DAFF) statistics]. During the initial stages of the program, progress was rapid, leading to a considerable reduction in the incidence of the disease in cattle by the mid-1960s. However, since then progress has stalled, and animal incidence of TB in 2004 was 0.33% (Good, 2007).

Bovine TB has been successfully eradicated from many developed countries, including Australia, most European Union member states, Switzerland, and Canada (De la Rua-Domenech et al., 2006). The failure of Ireland, the United States, and several other European Union member states to reach TB-free bovine herd status, coupled with the relatively high cost of the existing eradication programs, indicates a need to investigate alternative strategies. One approach, which could prove beneficial synergistically with current eradication schemes, would be genetic selection for increased resistance to TB in cattle. However, this strategy requires additive genetic variation to be present among animals in resistance to TB. Information is limited on whether genetic variation exists among cattle in their susceptibility to infection with *M. bovis*. Evidence at the genus level suggests that *Bos indicus* is less susceptible than *Bos taurus* to TB (Carmichael, 1941; Ram and Sharma, 1955). Furthermore, differential susceptibility to TB has been demonstrated in Zebu and Zebu cross cattle in Malawi (Ellwood and Waddington, 1972). In addition, there is evidence that certain family lines are more susceptible to TB than others. Petukhov et al. (1998) analyzed sire-offspring and daughter-dam relationships among 2,742 animals from 2 farms in Latvia and obtained heritability estimates ranging from 0.06 to 0.08 for resistance to TB; however, no indication was reported of the precision of the genetic parameters estimated in that study of limited size. Similarly, a heritability estimate of 0.06 to 0.16 has been published for Johne's disease, which is caused by a closely related mycobacterial species, *M. avium* ssp. *paratuberculosis* (Koets et al., 2000; Gonda et al., 2006).

The objective of the present study was to quantify the genetic variation present among Irish Holstein-Friesian dairy cattle in their susceptibility to TB. In this study, SICTT and postmortem factory tuberculous lesion data were used to assess *M. bovis*-PPD responsiveness and *M. bovis* infection status, respectively. The

results from this study will be useful in determining the feasibility of genetic selection of cattle for increased resistance to TB.

MATERIALS AND METHODS

Assessment of Cattle TB Status in Ireland

***M. bovis*-PPD Responsiveness.** Individual animal *M. bovis* infection status in Ireland is based primarily on the SICTT. In Ireland, each herd is subjected, at a minimum, to an annual test of all eligible animals in the whole herd, which is undertaken by authorized veterinary surgeons using published methodologies (European Economic Community, 1964; More and Good, 2006). The test involves injecting 0.1 mL of *M. bovis*-PPD antigen (1 mg/mL) into the neck of the animal. The reaction to *M. bovis*-PPD is compared with the skin reaction induced by injecting 0.1 mL of *M. avium*-PPD (0.5 mg/mL) to provide a measure of sensitization to the *M. avium* sp. individually and other nontuberculous *Mycobacterium* spp. highly prevalent in the Irish environment (Gormley et al., 2004). The SICTT skin change, the difference in response or reaction to the *M. bovis* and *M. avium* antigens, is measured 72 h after injection, whereby the size of the *M. avium*-PPD reaction is taken from that of the *M. bovis*-PPD (Martin et al., 2001). In the present study, animal susceptibility to *M. bovis*-PPD responsiveness was dichotomized as an "*M. bovis* standard reactor" [an animal with a skin change in response to the *M. bovis*-PPD inoculation 4 mm or greater than the reaction to the *M. avium*-PPD inoculation (Gormley et al., 2004)] or a "nonreactor" [an animal with a skin change in response to the *M. bovis*-PPD inoculation equal to the reaction to the *M. avium*-PPD inoculation (in this study)].

***M. avium*-PPD Responsiveness.** *Mycobacterium avium* ssp. *avium* is the causative agent of avian TB, a contagious disease of poultry and birds. Disease caused by *M. avium* ssp. *avium* is very uncommon in cattle, and the importance of exposure to the *M. avium* sp. and to subspecies in the context of the tuberculin test lies in the development of cross-sensitization to *M. bovis*-PPD (Pavlik et al., 2005). The importance of a response to *M. avium*-PPD in the SICTT is to differentiate the response to *Mycobacterium* spp. other than *M. bovis* (de la Rua-Domenech et al., 2006). Animal susceptibility to *M. avium*-PPD responsiveness was dichotomized as an "*M. avium* standard reactor" [an animal with a skin change in response to the *M. avium*-PPD inoculation 4 mm or greater than that of the *M. bovis*-PPD inoculation (Ameni et al., 2007)] or a "nonreactor."

Confirmed *M. bovis* Infection. Postmortem examination of every animal at slaughter for its fitness for

human consumption is an integral part of the ongoing bovine TB eradication scheme in Ireland. Lesions from *M. bovis* nonreactor animals at slaughter are forwarded to the Central Veterinary Research Laboratory. If *M. bovis* infection is confirmed through histopathology or culture, the source herd becomes restricted and an SICTT is scheduled under European Union and national legislation (Olea-Popelka et al., 2008). Animal susceptibility to confirmed *M. bovis* infection was dichotomized as “lesioned” (an animal with a confirmed tuberculous lesion after the SICTT) or “nonlesioned” (a nonreactor with no subsequently confirmed *M. bovis* infection).

In Ireland, herds with at least 1 TB-infected animal (i.e., based on the presence of a *M. bovis* standard reactor or abattoir lesion) are deemed to be “restricted.” Animal movement into and out of the restricted herd is controlled by national and European Union legislation until the herd is deemed “unrestricted,” which is achievable only by having 2 consecutive whole herd-tests [herd SICTT carried out on all eligible animals (ordinarily those >6 wk of age) on a specific date] with no standard or inconclusive *M. bovis* reactors (animals with a skin change in response to the *M. bovis*-PPD inoculation larger than 0 mm but less than 4 mm greater than the reaction to the *M. avium*-PPD inoculation).

Data Editing

A total of 1,345,036 animal SICTT results from 12,544 positive herd-tests (herd tests with at least 1 *M. bovis* standard reactor) in 5,164 herds, as well as 21,024 positive abattoir animal lesion records from November 1, 2000, to October 31, 2005, were obtained from the DAFF Animal Health Computer System and Factory Lesion Database, respectively. Movements of all animals in and out of herds are recorded in Ireland, and dates of movements ($n = 567,741$) as well as herd of origin and destination were obtained on all animal movements from January 1, 2000, to October 31, 2005, from the DAFF Cattle Movement and Monitoring System. Calving records and pedigree information were obtained from the Irish Cattle Breeding Federation database.

An attempt was made to include only animals that had a high likelihood of being exposed to *M. bovis*. Periods of infection within herd were identified and are herein referred to as episodes [Higgins et al., 2006; herd restrictions initiated by 2 or more *M. bovis* standard reactors, 1 of the animals being home bred, and terminated by 2 consecutive clear herd-tests (in this study)]. Furthermore, in Irish production systems cows and heifers tend to be reared in separate areas of the herd; hence, they are likely to experience different *M. bovis* infection pressures. Consequently, multiparous cows

and heifers were treated as 2 distinct contemporary groups.

To remove tuberculin tests that were atypical, that were not indicative of infection with *M. bovis*, or both, herd-tests with more than 5 standard reactors but with no observed factory lesion disclosed after postmortem examination (atypical herd; DAFF statistics) were deleted. The SICTT records from 853,355 animals (20,001 *M. bovis* standard reactors) across 6,271 herd-tests on 3,346 herds remained (53% of herds had one, 25% had two, 11% had three, 6% had four, and 5% had five or more positive herd-tests).

Animals that moved into the herd during or within 6 wk of the beginning of an episode were removed to maximize the likelihood of equalized, within-herd *M. bovis* exposure because it takes 3 to 6 wk postinfection for cattle to develop a positive reaction to the SICTT (De la Rua-Domenech et al., 2006).

Cow Data Sets. The initial data set consisted of 147,912 cows with calving records from 1,643 herds (3,260 positive herd-tests) within 1,944 episodes. Parity number was recoded into 6 classes: 1, 2, 3, 4, 5, and greater than 5. Cows of unknown parity and less than 15 mo of age ($n = 850$), and heifers ($n = 15,842$) calving within 6 wk after the herd-test (insufficient time had lapsed for these animals to develop a positive reaction following exposure to *M. bovis* within the cow cohort) or after the herd-test (these animals were tested as heifers, thus were not exposed to *M. bovis* within the cow category) were removed. Cows within episodes that lacked a home-bred *M. bovis* standard reactor were removed; 1,234 episodes containing 75,890 cows were discarded. A total of 6,016 cows with an age at calving outside 22 mo of the parity median were discarded. Nonreactors with subsequent inconclusive SICTT records, and nonreactors with later abattoir lesion records were removed ($n = 1,983$). Cows that moved into the herd within 6 wk of the herd-test ($n = 149$), and cows without a known sire ($n = 6,903$) were discarded, to generate a data set of 25,274 cow records. Separate data sets were generated from this data set to quantify the genetic variation among cows for susceptibility to *M. bovis*-PPD and *M. avium*-PPD responsiveness.

To estimate the genetic parameters of susceptibility to *M. bovis*-PPD responsiveness in cows, inconclusive *M. bovis* and *M. avium* reactors (animals with a skin change in response to the *M. avium*-PPD inoculation larger than 0 mm but less than 4 mm greater than the reaction to the *M. bovis*-PPD inoculation; $n = 3,755$) and standard *M. avium* reactors ($n = 1,558$) were deleted. The last record per cow within the data set ($n = 17,714$) was retained. Sires with fewer than 4 progeny

Table 1. Summary statistics of the *Mycobacterium bovis*-purified protein derivative (PPD) responsiveness (Mbo), *Mycobacterium avium*-PPD responsiveness (Mav), and confirmed *M. bovis* infection (cMbo) cow and heifer data sets

Item	Mbo		Mav		cMbo	
	Cow	Heifer	Cow	Heifer	Cow	Heifer
Animal records, n	14,013	7,712	10,410	5,665	13,791	7,959
Standard reactors or confirmed cases, n	1,329	682	1,045	649	630	399
Animal incidence, %	9.5	8.8	10.0	11.5	4.6	5.0
Herd-tests, ¹ n	821	399	515	281	624	388
Herds, n	415	192	285	138	378	206
Episodes, n	436	204	292	142	392	212
Animal pedigree file, n	34,511	22,195	26,641	17,080	25,324	15,603

¹Herd-tests refer to single intradermal comparative tuberculin test-positive herd-tests.

were removed (n = 1,095; 1,727 cow records). Episodes with no standard *M. bovis* reactor, and episodes with fewer than 10 cows were removed (n = 205; 1,974 cow records). Similarly, inconclusive *M. bovis* and *M. avium* reactors, standard *M. bovis* reactors, episodes with no standard *M. avium* reactor, and episodes with fewer than 10 animals were deleted to estimate the genetic parameters of susceptibility to *M. avium*-PPD responsiveness in cows. The final cow *M. bovis*-PPD and *M. avium*-PPD responsiveness data sets are summarized in Table 1.

Heifer Data Sets. The initial data set consisted of 61,801 heifers without calving records from 517 episodes in 487 herds. Heifers younger than 7.4 mo (6-mo-old females plus the 6-wk latent period; n = 15,757) and greater than 30 mo (n = 10,331) by their last herd-test within an episode were deleted. Heifers (n = 6,305) calving within 6 wk of, or after, the last herd-test within an episode were included in the heifer data set because they were exposed to *M. bovis* within the heifer cohort. Heifers (n = 9,022) within episodes (n = 137) that lacked a home-bred *M. bovis* standard reactor were deleted. Nonreactors with subsequent inconclusive SICTT records and nonreactors with later abattoir lesion records were discarded (n = 1,490). Heifers that moved into the herd within 6 wk (n = 31) of the SICTT, and heifers without a known sire (n = 3,068) were removed, resulting in a data set of 17,699 heifer records. Separate data sets were generated from this data set to quantify the genetic variation in heifers for susceptibility to *M. bovis*-PPD and *M. avium*-PPD responsiveness.

To estimate the genetic parameters of susceptibility to *M. bovis*-PPD responsiveness in heifers, inconclusive *M. bovis* and *M. avium* reactors (n = 1,388) and standard *M. avium* reactors (n = 1,032) were deleted. The last record per heifer within the data set (n = 10,013) was retained. Sires with fewer than 5 progeny were removed (n = 496; 803 heifer records). Episodes with no standard *M. bovis* reactor and episodes with fewer than

10 heifers were deleted (n = 122; 1,498 heifer records). Correspondingly, inconclusive *M. bovis* and *M. avium* reactors and standard *M. bovis* reactors, episodes with no standard *M. avium* reactor, and episodes with fewer than 10 animals were deleted to estimate the genetic parameters of susceptibility to *M. avium*-PPD responsiveness in heifers. The final heifer *M. bovis*-PPD and *M. avium*-PPD responsiveness data sets are summarized in Table 1.

Confirmed *M. bovis* Infection Data Sets. An additional data set was generated using data collected on the incidence of carcass lesions at abattoirs. A lesion-generated episode was defined as a herd restriction initiated by a confirmed *M. bovis* infection case (postmortem lesion disclosed or positive histopathology or culture results) within the animal category, and was terminated by 2 consecutive herd-tests with no confirmed case of *M. bovis* infection. The criterion of a home-bred TB case within each episode was not implemented because this requirement resulted in excessive loss of TB case records. Editing criteria for cows and heifers were otherwise the same as those for the SICTT data. Animals with no known sire or that moved into the herd within 6 wk of the herd-test, and nonlesioned animals with standard or inconclusive tuberculin results were deleted. Episodes lacking a confirmed case of TB and episodes with fewer than 10 animals were removed. The final cow and heifer TB data sets are summarized in Table 1.

Pedigree information on each animal was traced back 4 generations. The proportion of Holstein-Friesian genes was calculated; only animals that were at least 75% Holstein-Friesian were retained. Heterosis and recombination loss were calculated for each animal (VanRaden and Sanders, 2003).

Data Analysis

Linear animal models (**LAM**) use all relationships and are most appropriate for estimating variance com-

ponents because the likelihood is exact; however, the variance of a binary trait is dependent on the incidence; thus, the basic assumption that mean and variance are independent is violated. Threshold animal models (TAM) take the binary nature of disease traits into account and use all relationships, but are less appropriate for estimating variance components because the likelihood is an approximation. Threshold sire models (TSM) are generally favored because they are computationally less intensive than TAM (Kadarmideen et al., 2000). However, TSM ignore the relationship between dam-daughter pairs, and thus may lead to upwardly biased estimates. To determine the most appropriate model, LAM, TSM, and TAM were used to estimate the variance components for susceptibility to *M. bovis*-PPD and *M. avium*-PPD responsiveness and confirmed *M. bovis* infection with ASREML (Gilmour et al., 2008). In general, TAM, which assume errors following a logistic distribution rather than a normal distribution, converge more easily. Because there is no evidence that the trait measure liabilities in this study followed a normal distribution, threshold models assuming a logistic underlying liability variable (L ; which determined the categorical outcomes of SICTT, such that $L \leq 0$ corresponds with $Y = 0$, and $L > 0$ corresponds with $Y = 1$) were fitted using a logit link function. The residual variances were set to $\pi^2/3$ (Guerra et al., 2006). The fixed effects considered for inclusion in the models were herd-episode, year of herd-test, month of herd-test, year of herd-test $\times$ month of test interaction, proportion of Holstein-Friesian genes, heterosis, recombination loss, parity, age (months), age nested within parity, year of calving, season of calving, the year of calving $\times$ season of calving interaction, and month of calving. The fixed effects added to the respective models differed by animal category and by trait measure analyzed. The fixed effects of herd-episode, year of herd-test, month of herd-test, and the year of herd-test $\times$ month of test interaction were added to all models tested. The fixed effect of month of calving was also added to the *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection cow models; parity was added to the *M. avium*-PPD responsiveness cow model; and the Holstein-Friesian breed fraction was added to the *M. bovis*-PPD responsiveness heifer model. All fixed effects were included as class variables. Additional analyses were undertaken in which the random effects of dam of animal and dam lineage were also included in the models.

The observed binary scale LAM heritability estimates were transformed to the underlying liability scale by using the formula of Robertson and Lerner (1949), to make comparisons with the estimates from the TAM. Sire EBV were computed and Spearman rank cor-

relations were estimated between the EBV from the different models. The significance of the correlation coefficients was tested using Fisher's Z -transform test, which compared the obtained coefficients with unity. A series of bivariate analyses using linear sire models were used to estimate phenotypic and genetic covariances among trait measures. It was not possible to estimate the environmental covariance between *M. bovis*-PPD responsiveness in heifers and cows; animals can only have a single positive SICTT record because they are culled after a standard *M. bovis* reaction. Similarly, it was not possible to estimate the environmental covariance between *M. bovis*- and *M. avium*-PPD responsiveness in heifers and cows; an animal cannot have a record for both trait measures on any given herd-test. The environmental covariance between 2 trait measures in the bivariate analysis was fixed to be 3 times the respective sire covariance. Only environmental covariance, and environmental and phenotypic correlations were estimated for *M. bovis* infection and confirmed *M. bovis* infection in cows. The standard error of the correlation coefficients between *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection were close to the upper bound; therefore, the likelihood ratio test (LRT) between nested models was used to determine whether the correlations differed significantly from 1. The likelihood ratio test between nested models was also used to determine whether the genetic correlation between the same trait measures in both cows and heifers differed significantly from 1, or whether genetic variances in both cows and heifers differed significantly from each other.

RESULTS

Mycobacterium bovis-PPD and *M. avium*-PPD responsiveness, and confirmed *M. bovis* infection incidences for cows and heifers are presented in Table 1.

Heritability Estimates

Genetic parameters for the different trait measures from the different models are summarized in Table 2. There was no significant maternal genetic or common environmental variance for any of the trait measures analyzed ($P > 0.05$). Model convergence was not possible once the maternal lineage effect was added to the respective models. Consequently, the maternal lineage effect was removed from subsequent analyses.

Estimated heritabilities for susceptibility to *M. bovis*-PPD responsiveness from the 3 models ranged from 0.040 to 0.274 in cows and from 0.038 to 0.276 in heifers. The TAM heritability estimates of 0.144 in cows and 0.119 in heifers most closely resembled the binary

Table 2. Genetic standard deviation (σ_a) and heritability estimates (subscript is the SE of the estimate) of susceptibility to *Mycobacterium bovis*-purified protein derivative (PPD) responsiveness (Mbo) in cows and heifers, *Mycobacterium avium*-PPD responsiveness (Mav), and confirmed *M. bovis* infection (cMbo) in cows from the linear animal model (LAM), threshold animal model (TAM), and threshold sire model (TSM) in the 2 animal categories¹

Item	Parameter	LAM	h_L^2	TAM	TSM
Cows					
Mbo	σ_{β}	0.054***	—	0.742***	0.985***
	h^2	0.040 _{0.012}	0.122 _{0.037}	0.144 _{0.028}	0.274 _{0.074}
Mav	σ_{β}	0.052*	—	0.687***	0.950**
	h^2	0.039 _{0.014}	0.114 _{0.040}	0.125 _{0.030}	0.257 _{0.077}
cMbo	σ_{β}	0.029*	—	0.842***	1.102**
	h^2	0.021 _{0.010}	0.097 _{0.047}	0.177 _{0.041}	0.338 _{0.111}
Heifers					
Mbo	σ_{β}	0.049*	—	0.668**	0.987**
	h^2	0.038 _{0.018}	0.119 _{0.057}	0.119 _{0.039}	0.276 _{0.101}

¹Heritability estimates from the linear animal model were transformed to the underlying liability scale (h_L^2). The test of significance for estimated variance components was based on the *t*-test, which determines whether the effect size of variance components differs significantly from zero.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

transformed liability estimates (Table 2; h_L^2). Spearman rank correlations among sire EBV for susceptibility to *M. bovis*-PPD responsiveness between the LAM and the TAM in cows and heifers were 0.986 and 0.992, respectively; nonetheless, coefficients were significantly different from unity ($P < 0.001$). Spearman rank correlations between sire EBV for susceptibility to *M. bovis*-PPD responsiveness from the TAM and the TSM were lower; coefficients of 0.913 and 0.934 were estimated in cows and heifers, respectively, and both differed significantly from unity ($P < 0.001$). The estimated heritabilities for susceptibility to *M. avium*-PPD responsiveness in cows from the 3 models ranged from 0.039 to 0.257. The TAM heritability estimate of 0.125 most closely resembled the binary transformed liability estimates. The genetic variance component for *M. avium*-PPD responsiveness susceptibility in heifers was not different ($P > 0.05$) from zero. Heritability estimates for cow susceptibility to confirmed *M. bovis* infection from the 5 models ranged from 0.021 to 0.338. The TAM heritability estimates of 0.177 most resembled the binary transformed liability estimates. The genetic variance component for confirmed *M. bovis* infection susceptibility in heifers was not different ($P > 0.05$) from zero.

Correlations

The correlations between the different trait measures are summarized in Table 3. The positive genetic correlation ($r_g = 0.53$; SE = 0.18) estimated for susceptibility to *M. bovis*-PPD responsiveness between cows and heifers was different ($P < 0.05$) from unity. The genetic variance components for susceptibility to *M.*

bovis-PPD responsiveness in cows and heifers were equal. A positive genetic correlation ($r_g = 0.842$; SE = 0.2130) was estimated between *M. bovis*-PPD and *M. avium*-PPD responsiveness in cows, which did not differ ($P > 0.05$) from unity. The genetic variance components for susceptibility to *M. bovis*-PPD and *M. avium*-PPD responsiveness in cows were equal ($P > 0.05$). A very strong positive genetic correlation ($r_g = 0.999$; $P > 0.05$) was estimated between *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection in cows, which did not differ from unity. The genetic variance components for susceptibility to *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection in cows did not differ ($P > 0.05$). High positive environmental ($r_e = 0.994$; $P < 0.05$) and phenotypic ($r_p = 0.994$; $P < 0.05$) correlations, which differed from unity, were estimated between *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection in cows, indicating quite analogous measurement error, and that the *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection, respectively, were similar measures.

DISCUSSION

Genetic Parameters

Significant genetic variability for susceptibility to confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness exists within the Irish Holstein-Friesian dairy herd. When the binary transformed liability heritability estimates were compared against the TSM and TAM, sire estimates from the TSM were biased upward because the relatedness between dam-daughter pairs was ignored. The TAM was therefore the most appropriate model for the estimation of the genetic parameters

Table 3. Genetic (r_g), environmental (r_e), and phenotypic correlation (r_p) coefficients (subscript is the SE of the coefficient) between susceptibility to *Mycobacterium bovis*-purified protein derivative (PPD) responsiveness (Mbo) in cows and heifers, *M. bovis*-PPD responsiveness and *Mycobacterium avium*-PPD responsiveness (Mav) in cows, and *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection (cMbo) in cows from the bivariate linear sire models¹

Item	Cows-Mbo						
	r_g	r_1	$v_{1=2}$	r_e	r_1	r_p	r_1
Heifers-Mbo	0.532 _{0.182}	**	NS	—	—	—	—
Cows-Mav	0.842 _{0.213}	NS	NS	—	—	—	—
Cows-cMbo	0.999 _{UB}	NS	NS	0.994 _{UB}	***	0.994 _{UB}	***

¹The test of significance was based on the likelihood ratio test (LRT), which determines whether correlations differ significantly from 1 (r_1) or whether genetic variances of the different trait measures differed significantly from each other ($v_{1=2}$). The standard error of the correlation coefficients between *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection were close to the upper bound (UB); therefore, the LRT was used to determine whether the correlations differed significantly from 1.

** $P < 0.01$; *** $P < 0.001$; NS = $P > 0.05$.

for susceptibility to confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness. The TAM heritability estimate for confirmed *M. bovis* infection susceptibility was greater than that for *M. bovis*-PPD responsiveness in cows, which was expected because the introduction of the SICTT test and slaughter policy in 1954, when animal incidence was at 17%, would have exerted selection pressure on the *M. bovis* response to the SICTT, resulting in the possible loss of genetic variation. Similar heritabilities were estimated for susceptibility to *M. bovis*-PPD responsiveness in cows and heifers. Significant genetic variation for susceptibility to confirmed *M. bovis* infection was not observed in heifers; this was probably a result of insufficient records, which generated a larger standard error (0.067), because the genetic standard deviation of 0.030 was larger than the estimate in cows. The heritability estimates for susceptibility to confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness were similar to those in the literature for TB resistance (0.06 to 0.08, defined by clinical, allergic, and histological methods; Petukhov et al., 1998) and other related disease traits such as paratuberculosis (0.06; Koets et al., 2000). A heritability of 0.48 for TB resistance in red deer (*Cervus elaphus*) has been estimated after experimental exposure (Mackintosh et al., 2000). Theoretically, the true heritability for susceptibility to *M. bovis*-PPD responsiveness in Holstein-Friesians may be closer to the estimate in red deer because inaccuracies resulting from imperfect SICTT sensitivities and specificities across herds (De la Rua-Domenech et al., 2006) may be biasing heritability estimates downward.

The moderate genetic correlation observed between *M. bovis*-PPD responsiveness in cows and heifers indicates that the trait measure may be controlled to some degree by the same genes in the 2 female animal categories. The positive genetic correlation, with an

inclination of almost unity observed between susceptibility to confirmed *M. bovis* infection and *M. bovis*-PPD responsiveness in cows, indicates the high degree to which the same or closely positioned genes influence both of these measures, and that direct selection to reduce susceptibility to *M. bovis*-PPD responsiveness in Holstein-Friesian dairy cows will indirectly select for increased resistance to confirmed *M. bovis* infection. Genetic merit for *M. bovis*-PPD responsiveness therefore provides an indirect measure of genetic merit for confirmed *M. bovis* infection within the national cow herd.

The empirical question remains, however, whether selection against *M. bovis*-PPD responsiveness could impair the ability of cattle to mount a detectable response to *M. bovis*-PPD, and hence the efficacy of the national eradication program to remove infected animals before they become a source of *M. bovis* for other animals. Similarly, there has been apprehension that selection for reduced SCC (an indicator trait of clinical mastitis) may impair the immune function. However, no such trend has been associated with low SCC. On the contrary, cows with the lowest observed SCC have the lowest risk of mastitis (Rupp and Boichard, 2003). Nonetheless, studies will be required to monitor the consequences of selection against *M. bovis*-PPD responsiveness in Irish dairy cattle.

Significant genetic variability for susceptibility to *M. avium*-PPD responsiveness also exists within the national Holstein-Friesian dairy cows. This result was expected because, although *M. avium* is more or less pathogenically inert (Pavlik et al., 2005), its infectivity may have been hypothetically attenuated in cattle through its shared evolutionary history (Brosch et al., 2001) with pathogenic species such as *M. bovis* and *M. avium* ssp. *paratuberculosis*. Significant genetic variability for susceptibility to *M. avium*-PPD responsiveness

was not observed in heifers, most probably as a result of the absence of variance (the genetic SD in cows was 2.6 times larger than the estimate in heifers). Heritability estimates in cows were similar to those estimated for susceptibility to *M. bovis*-PPD responsiveness and confirmed *M. bovis* infection, and to that reported previously for susceptibility to paratuberculosis (0.06; Koets et al., 2000) caused by *M. avium* ssp. *paratuberculosis*.

The positive genetic correlation observed between susceptibility to *M. avium*- and *M. bovis*-PPD responsiveness indicates that selection for increased resistance to *M. bovis*-PPD responsiveness will be indirectly selecting for resistance against members of the *M. avium* and *M. tuberculosis* complexes, such as *M. avium* ssp. *paratuberculosis* and *M. tuberculosis*, respectively. Furthermore, selection for resistance to *M. bovis*-PPD responsiveness may be indirectly selecting for resistance to other infections and so better general health, considering that it has been demonstrated that the cytotoxic T-lymphocyte epitope of the mycobacterial heat shock protein 65 not only shares remarkable homology among mycobacterial species, but also that it is identical for a large number of pathogenic bacteria, such as the ethological agents of brucellosis, *Brucella abortus*, and chronic mastitis, *Staphylococcus aureus*, in cattle (Charo et al., 2001). Nonetheless, the immune system is known to modulate different effectors and pathways according to the pathogen type (Rupp and Boichard, 2003). This raises the question of whether resistance against *M. bovis* infection is independent or antagonistically associated with resistance against other pathogens, for example, the extracellular helminth parasite *Ostertagia ostertagi*. Such associations will have to be ascertained if selection against *M. bovis*-PPD responsiveness is to be included in breeding programs.

Methodological Issues

Over the years, several herd- and animal-level factors have been associated with increased risk of failing the SICTT. In this study, all the major risk factors were either accounted for methodologically or adjusted for in analyses. The greater risks associated with non-singleton restrictions (Olea-Popelka et al., 2004) and cows failing the SICTT (Martin et al., 2001) were nullified by including only episodes with 2 or more standard reactors in the analyses and the generation of distinct cow and heifer episodes. The critical risk factors of farm location, herd size (Martin et al., 2001), and prevalence of TB (Olea-Popelka et al., 2004), as well as the temporal differences in reactor risk both between and within years (Martin et al., 2001), were adjusted for by fitting the effects of defined episodes, as well as month and year of herd-test, in all models.

An important environmental factor is the degree of exposure to *M. bovis* (More and Good, 2006). Accordingly, a substantial attempt was made in the present study to maximize the opportunity of exposure to *M. bovis*, and hence for trait expression in all animals analyzed. Specific cow and heifer episodes were generated because cows and heifers are generally managed as separate cohorts on Irish dairy farms and were thus unlikely to experience the same *M. bovis* infection pressures. Only episodes with at least 2 standard reactors were included in the analysis because the probability of 2 false positives is minimal (More and Good, 2006), guaranteeing at least 1 true positive per episode. The requirement for 1 home-bred standard reactor ensured that exposure was not transient exposure from the entry of an *M. bovis*-infected animal. Furthermore, animals that moved into the herd within 6 wk of the test were removed because insufficient time had lapsed to develop a positive reaction to the SICTT post-exposure; hence, the animals were not exposed or were nonexposed within the episode.

The success of any genetic epidemiology study depends on the accuracy of the diagnostic test (Gonda et al., 2006), which is a function of its specificity (ability to correctly identify uninfected animals) and sensitivity (ability to correctly identify infected animals). Post-mortem confirmation of *M. bovis* infection may appear to be the most accurate measure for susceptibility to *M. bovis* infection. However, TB eradication is in the latter stages in Ireland, whereby clinical disease rarely presents itself, rendering postmortem inspections far less sensitive than the SICTT. The specificity of the SICTT is 99.9% (De la Rua-Domenech et al., 2006); thus, the likelihood of detecting a false negative is very low. Nonetheless, the SICTT has an imperfect test sensitivity of 90.9% (More and Good, 2006). Furthermore, the accuracy of the SICTT is not a fixed test characteristic because test results are influenced by the stage of the disease and underlying trait diagnoses (De la Rua-Domenech et al., 2006). In this study, to rule out latent infections and animals in a state of anergy, cattle that moved into the herd within 6 wk of the TB test and nonreactors with subsequently confirmed *M. bovis* infection were removed from the analyses. A high diagnostic threshold of the standard *M. bovis* reactor was set, and standard and inconclusive *M. avium* reactors were removed from the analysis of *M. bovis*-PPD responsiveness, minimizing the likelihood of false positive or negative results, and hence accurate identification of genetically relevant phenotypes. Furthermore, in the estimation of variance components, one is interested in the genetic merit of family groups; hence, as within-herd family size increases, herd family-level sensitivity also increases, hypothetically making the SICTT a

good “family test” for the genetic evaluation of cattle for susceptibility to *M. bovis*-PPD responsiveness. Nonetheless, SICTT inaccuracies may have influenced the genetic variance in this study, deflating heritability estimates, indicating that the response to selection for *M. bovis*-PPD responsiveness may be hypothetically greater than that predicted from the parameter estimates in this study.

Analyses of binary traits such as susceptibility to *M. bovis* infection causes statistical challenges because the variance of the trait will be dependent on the incidence; the nearer the incidence is to 50%, the lesser this dependence becomes, whereas generalized mixed models are preferable when incidences lie outside 10 to 90% (Falconer, 1981). Threshold models have been shown to be theoretically better and are well accepted for the estimation of variance components of binary traits (Kadarmideen et al., 2000; Guerra et al., 2006). As expected, the LAM heritability estimates obtained in this study were lower than those from the threshold models because transformation to the underlying liability scale reduces the variance of measurement error (Falconer, 1981). Traditionally, TSM are favored, because TAM are computationally more unstable; therefore, reaching convergence is difficult (Kadarmideen et al., 2000). However, much depends on the relatedness of animals (i.e., how many dam-daughter pairs are present) because TSM ignore these, which may lead to an upwardly biased estimate. To determine the most appropriate heritability estimates, categorical analysis was conducted using both TSM and TAM. The appropriateness of each estimate was determined by its comparability with the binary transformed liability estimate. Heritability estimates from the TSM were biased upward, which most likely resulted from the absence of a dam effect in these models. The TAM was therefore the most applicable model for the disease trait measures in this study. An animal logistic threshold model has been used successfully to estimate the heritability of claw and foot disorders in cows (Koenig et al., 2005). Nonetheless, Guerra et al. (2006) reported that LAM and TAM models generate sire estimates with similar rankings. The LAM and TAM EBV for susceptibility to *M. bovis*-PPD responsiveness in cows and heifers were near perfectly correlated, indicating that either LAM or TAM could be used for the analysis of susceptibility to *M. bovis*-PPD responsiveness at a national level, although available computing facilities would favor LAM.

Implications for Genetic Improvement

Significant genetic variation for *M. bovis*-PPD responsiveness exists in the national dairy herd, indicating

that genetic improvement is possible, provided appropriate evaluation and selection protocols are applied. However, to determine the feasibility of extending the Irish breeding objective to include susceptibility to *M. bovis*-PPD responsiveness, genetic and phenotypic correlations with traits of the selection criterion need to be quantified so that *M. bovis*-PPD responsiveness can be weighted appropriately within the Irish economic breeding index. It is also of interest to determine whether significant genotype $\times$ environment interactions exist for susceptibility to *M. bovis*-PPD responsiveness across varying production, management, regional, and epidemiologically defined environments, and if so, whether the provision of sire EBV to farmers may be more applicable to the Irish situation.

CONCLUSIONS

This study demonstrated that significant genetic variability for susceptibility to confirmed *M. bovis* infection exists within the Irish Holstein-Friesian dairy herd. Selection for increased resistance to *M. bovis*-PPD responsiveness (as measured by the SICTT) will indirectly reduce susceptibility to *M. bovis* infection, and hence the incidence of TB within the national dairy herd, which will synergistically benefit existing eradication programs. The large, high-quality DAFF (Animal Health Computer System, Factory Lesion, and Cattle Movement and Monitoring System) and Irish Cattle Breeding Federation databases with linkable herd- and animal-level records, the methodological and analytical equalization of risk of failing the SICTT and exposure to *M. bovis*, the accurate identification of genetically relevant phenotypes, and the application of statistically appropriate TAM in this study each contributed to calculation of the most credible heritability and robust parameter estimates to date for susceptibility to *M. bovis*-PPD responsiveness in cattle. These data are collected routinely within the national TB eradication program; therefore, it should be possible to develop breeding programs to select against confirmed *M. bovis* infection, via the SICTT. Sire rankings from the LAM and TAM were similar, indicating that both models are suitable for the genetic evaluation of susceptibility to *M. bovis*-PPD responsiveness in the national dairy herd. However, limitations on existing computing resources would favor the use of the LAM.

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