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Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*

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ABSTRACT

Anecdotal evidence points to genetic variation in resistance of cattle to infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (BTB), and published experimental evidence in deer and cattle suggests significant genetic variation in resistance and reactivity to diagnostic tests. However, such genetic variation has not been properly quantified in the United Kingdom dairy cattle population; it is possible that it exists and may be a factor influencing the occurrence of BTB. Using models based on the outcome of the process of diagnosis (ultimate fate models) and on the outcome of a single stage of diagnosis (continuation ratio models, herd test-date models), this study shows that there is heritable variation in individual cow susceptibility to BTB, and that selection for milk yield is unlikely to have contributed to the current epidemic. Results demonstrate that genetics could play an important role in controlling BTB by reducing both the incidence and the severity of herd breakdowns.
Key words: resistance to infection, bovine tuberculosis, heritable variation, controlling bovine tuberculosis

INTRODUCTION

Bovine tuberculosis (**BTB**) is a serious cattle disease that arises from infection by *Mycobacterium bovis*. It is widespread in the United Kingdom, Ireland, Africa, parts of Asia, and some Middle Eastern countries and is also found in a few US states and in New Zealand. *Mycobacterium bovis* is spread primarily by aerosolized respiratory secretions containing bacilli, from infected to uninfected animals, and has a broad range of animal hosts, which complicates its control. It is generally accepted that cattle in Great Britain (**GB**) are infected from other cattle or from wildlife (principally badgers, *Meles meles*). By the mid-1980s, a UK government testing and slaughter program made compulsory in 1950 reduced the national incidence of BTB in cattle to a very low level. Despite the continuation of this program, the incidence of BTB has risen in the last 20 years. In GB in 2008, approximately 40,000 suspect animals were identified and slaughtered (www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/animalh/tb/detailedstats/detailedstats.htm). In addition to the financial and social costs incurred by farmers, cattle testing, compensation, and ancillary work currently cost around $150 million per year (www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/tb/stats/other.htm). If the national incidence continues to rise at the current rate, it is estimated that from 2004 to 2011, the total economic costs associated with BTB will reach $1.65 billion (Defra, 2004).

Within GB, there has been much debate about the relative importance of different sources of infection for cattle herds and of effective methods to limit maintenance and ongoing spread of BTB in cattle. The importance of infected cattle movements (Gilbert et al., 2005) and, more generally, of improved cattle controls (Bourne, 2007) have been highlighted when considering strategies for improved control. Current evidence for BTB persistence suggests that several control measures could be implemented, including changes to husbandry and farm management practices (White et al., 2008). A range of methods to limit contact between badgers and cattle, including badger removal and biosecurity measures, have been considered in detail. Although proactive badger removal is associated with a significant decrease in BTB incidence in cattle (Griffin et al., 2005; Donnelly et al., 2006), concern has been raised in GB about the potential for adverse effects (specifically

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increased BTB incidence in cattle) caused by badger perturbation following badger removal (Donnelly et al., 2006). Development of BTB vaccines for badgers and cattle has the potential to contribute to BTB eradication, and progress has been made with regard to badgers (e.g., Lesellier et al., 2009). It is anticipated that an injectable badger vaccine will be licensed in 2010 and an oral vaccine by 2014 at the earliest (www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/tb/research/vaccine.htm). However, vaccination is a long-term strategy and it is likely to take several years before a reduction in BTB in cattle is observed due to vaccination of badgers, and other measures to reduce the incidence of BTB will still be required.

Useful genetic variation has been found for several diseases in cattle. For example, data on respiratory disease in Norwegian Red calves was analyzed and the researchers concluded that reasonably precise genetic evaluation for resistance to respiratory disease is feasible (Heringstad et al., 2008). The heritability of antibody response to paratuberculosis has been estimated as 0.10, prompting the conclusion that data from diagnostic tests could be used in breeding programs (Hinger et al., 2008). To date there have been only 2 published studies of genetic variation in resistance to *M. bovis* in livestock. In a study of farmed red deer, offspring of stags that displayed differing responses to experimental challenge from *M. bovis* were bred, and by comparing the response of offspring with that of their sires, a strong genetic basis to resistance to BTB was estimated (heritability = 0.48 ± 0.096; Mackintosh et al., 2000). In recent Irish work (Bermingham et al., 2009), estimated heritabilities for susceptibility to *M. bovis*-purified protein derivative (**PPD**) responsiveness (a proxy for BTB infection) ranged from 0.040 to 0.274 in dairy cows and from 0.038 to 0.276 in dairy heifers. The BTB testing regimen in Ireland is different from that in GB and, more importantly, Irish dairy cattle are managed very differently compared with cattle in GB. The Irish pasture-based system results in lower milk yields with concomitant lower prevalence of associated diseases (www.teagasc.ie/research/reports/dairyproduction/5403/eopr-5403.asp). Estimates of genetic variation from an Irish dairy population cannot therefore be assumed to hold for the more intensively managed GB population.

The objective of this work was to estimate the extent of genetic variation in susceptibility or resistance to BTB in the GB dairy cattle population and determine whether genetic evaluation can contribute to a reduction in BTB incidence. In addition, the genetic association with milk yield was investigated to determine if selection for increased milk yield resulted in an increase in BTB.

MATERIALS AND METHODS

Tuberculin Skin Testing in GB Cattle

The data used in this study were the results of diagnostic tests conducted in dairy herds in GB from 2000 to 2007, including the single intradermal comparative cervical test (**SICCT**) in live animals, and gross inspection [for abnormal pathology (lesions), suggestive of BTB infection] and bacteriology on animals following slaughter. The salient features of the testing regimen in relation to this study are as follows. The SICCT involves separate intradermal injection of *Mycobacterium avium* and *M. bovis* PPD, followed by interpretation 72 h later of any observed skin thickening. *Mycobacterium avium* PPD is used to assess exposure of tested cattle to environmental mycobacteria and thus minimize the risk of false-positive results. The precise usual interpretation of the tests is a standard procedure applied internationally across the European Union (de la Rua-Domenech et al., 2006). On the basis of the SICCT result, an animal is classified as 1) a nonreactor (**NR**), in which the response to *M. bovis* is judged in a defined way to be less than or equal to that of *M. avium*, and hence indicative of freedom from BTB; 2) an inconclusive reactor (**IR**), in which the reaction to *M. bovis* exceeds that to *M. avium* but not to a degree considered to be clearly indicative of BTB; or 3) a reactor (**R**), in which the response is deemed to be clearly indicative of disease. Discovery of an R during testing triggers a new tuberculosis incident (or breakdown) and prompts further tests at prescribed 60-d intervals. All R animals are valued and sent for slaughter. The herd is placed under movement restrictions and testing continues until 2 consecutive negative herd tests (1 negative herd test in the case of an unconfirmed incident) are obtained, at which time the restrictions are lifted and the herd is considered officially free from BTB. In GB, during the study period, all animals classified as IR were retested at 60-d intervals to resolve their status and, if they remained IR after 2 consecutive retests, they were culled as R. In GB, all cattle destined for human consumption are inspected at the abattoir for gross pathology typical of BTB and other diseases. Known R and IR are given a more detailed inspection at the abattoir, and samples of tissue with gross pathology from a sample of reactors from each breakdown or a pool of prescribed lymph nodes in the case of animals that have no pathology typical of BTB are sent for bacteriological and histological analysis. Breakdowns in which either macroscopic lesions typical of BTB have been observed in one or more reactors or there has been bacteriological confirmation of infection with *M. bovis* has been isolated from one or more animals are

described as confirmed breakdowns for the purposes of BTB control. Surveillance testing of herds outside testing of breakdowns is conducted nationally at an intensity reflecting the perceived risk of disease in the region. During the period of interest, the blood-based γ-interferon test was not used as a part of routine herd surveillance in GB, although its use in BTB control has been introduced in some prescribed circumstances since October 2006.

Data Extraction and Editing

Data on animals tested between 1995 and 2007 were extracted from 2 complementary but previously unlinked databases. The first was the Defra VetNet database, which maintains summary GB TB surveillance data on all herd tests and more detailed testing records for breakdown herds. For the latter, VetNet records details about the herd, the breakdown and all cows found to have tested IR or R together with the difference measurement between the skin reactions to *M. avium* and *M. bovis* tuberculins leading to this categorization. Note that individual animals testing NR at a skin test are completely unknown and unrecorded in the VetNet database unless taken as dangerous contacts (animals that are not deemed test reactors but are considered exposed to infection and removed) when the difference measurement is again recorded.

The second database was compiled from commercial companies recording performance in dairy herds. This database came from 2 sources; National Milk Records plc (Cirencester, Gloucestershire, UK) and the Centre for Dairy Information performance database managed by Holstein UK (Scotsbridge House, Rickmansworth, Hertfordshire, UK). Performance tests are carried out on lactating animals at intervals of around 28 d, and each database contains records on all milking cows within a herd present on the date of the test.

The databases were matched on the animal's official identification (ear tag). Given a match between an animal in the VetNet database and the industry databases on a performance test-day close to skin test date, it was possible to infer the contemporary animals that were present at the skin test but were NR (and were therefore not in the VetNet database).

The initial matching process identified in excess of 450,000 records on animals contained on VetNet and their contemporaries, although this included more than 1 record per cow. Several data edits were made to improve the quality of the records that were analyzed. Initial edits were on information quality. All cattle other than Holstein-Friesian cows were removed (this combined gene pool comprises 95% of all dairy cows in the UK), and herds with fewer than 5 cows were removed. Animals classed as dangerous contacts were also removed because they were few in number and their skin test results were frequently not recorded. The following editing of breakdowns was made for epidemiological reasons:

(i) Over time, herds may experience more than one breakdown. Only the first breakdown in a herd during the period of interest, judged from within the extracted data set, was included.
(ii) After applying edit (i), breakdowns occurring earlier than 2000 were removed (i.e., all records associated with that breakdown were removed). Because the data set screened started in 1995, and the maximum length of the testing cycle in the UK is 4 yr, all herd breakdowns included in the analysis were from herds that had been clear for at least 4 yr.
(iii) Breakdowns that had not resolved after 2 yr were removed.
(iv) Breakdowns with fewer than 2 R cows were removed to ensure that the breakdown was not due to a single, possibly imported, cow.
(v) For cows appearing in more than one breakdown, records apart from the first were removed from the data.

A screen was made to identify exposed groups, in particular to address the possibility that first-lactation animals may have been subject to a different exposure risk to those in later lactations because of their management. Therefore, the following additional edits were applied:

(vi) If at least 90% of R cows (class 7, Table 1) occurred within a single age group, then all other groups were deleted, on the assumption that the breakdown originated from a point source within a cohort.
(vii) If no first-lactation cows were identified as R (class 7, Table 1) within the breakdown, then the entire first-lactation cohort was deleted, on the assumption that the epidemic originated within the milking herd.

Following this editing, records remained on 68,497 cows in 818 herds, with an average of approximately 80 cows per herd (Table 1). Pedigrees for 5 generations were then extracted using the National Milk Records and Holstein UK pedigree databases for all remaining cows with breakdown data or inferred breakdown data (NR). This resulted in a total of 228,508 animals being included in the pedigree.

Table 1. Number and percentage of cows in each of the 7 classes defined by the passage of the cow through the breakdown as well as the scoring of the cow in the ultimate fate models (models C and D)[1]

Class	Ultimate fate models	Test 1	Test 2	Test 3	Cows	
					n	%
1	0	NR			58,086	84.8
2	0	IR	NR		4,444	6.5
3	0	IR	IR	NR	986	1.4
4	1	IR	IR	IR	183	0.3
5	1	IR	IR	R	118	0.2
6	1	IR	R		410	0.6
7	1	R			4,270	6.2
Total					68,497	

[1]NR = nonreactor; R = reactor; IR = inconclusive reactor.

Models Fitted

A range of models was fitted as described below. The first 2 models are termed "ultimate fate" models, which are simple summary statistics of all the test outcomes over the whole breakdown period. Further models (the continuation ratio model and the herd test-date model) were then fitted to model the repeated testing that was carried out. Fitting of all models was done using REML in the software package ASReml (Gilmour et al., 2006).

Ultimate Fate Model C (Culled). The data from the series of skin tests were summarized by defining the ultimate fate ($\mathbf{u_C}$) of the cow following interpretation of the SICCT result during the breakdown: $u_C = 1$ if a cow has been culled at the completion of the breakdown irrespective of whether it was culled as an R or as a multiple IR; and $u_C = 0$ otherwise (Table 1). Such an all-or-none trait can be treated as continuous on an underlying scale of liability, a quantitative measure of how likely an individual is to be diseased. Models fitted to the binary variable "culled as a reactor or not" estimated effects on the underlying liability scale using a complementary log-log link function. Estimates of heritability and other variance components are, in principle, independent of prevalence within different herd breakdowns. Before first calving, cows are managed separately from the milking herd. As first-lactation animals may therefore have experienced a different exposure risk compared with cows in later lactations, cows were grouped into milking heifers and later lactations. The fixed effects included were herd breakdown, lactation group, interaction between herd breakdown and lactation group, and month of test. Age (in days) and milk yield were included in the model as orthogonal polynomials of degree 2. A random genetic effect was fitted for each animal; that is, a full animal model was fitted to the data.

In addition, a bivariate analysis was carried out including the daily milk yield on the test date closest to the skin test date as a separate but correlated trait. The model for milk yield included age at milk recording and stage of lactation in days, both modeled as quadratic polynomials.

Ultimate Fate Model D (Diagnosed Following Culling). This model was similar to ultimate fate model C, except that the trait was defined not only by culling (following interpretation of the SICCT result), but also by whether there was gross pathological or bacteriological evidence following culling consistent with BTB infection. Therefore, in model D, ultimate fate ($\mathbf{u_D}$) = 1 only if $u_C = 1$ *and* evidence from either gross pathology or bacteriology following culling and slaughter suggestive of BTB infection, otherwise $u_D = 0$. Because the additional evidence was not available for all animals, further data editing was required, resulting in records on 18,339 cows in 221 herds. A total of 76,284 animals were included in the pedigree. The effects fitted were as for model C.

Two additional models were introduced to model the repeated testing that was carried out within the breakdown. We call these the continuation ratio model and the herd test-date model.

Continuation Ratio Model. During a breakdown, the result of each test is NR, IR, or R, and we regard IR as intermediate in severity between NR and R. An R result ends testing for that animal because the animal is culled; an IR result leads to further testing (until the third IR). In total, there are 7 different outcomes, or classes of cow, from the testing regimen in a herd breakdown (see Table 1). For example, an animal may be clear on the first test (class 1), she may be an IR on the first test but clear on the second (class 2), or she could be judged an IR at all 3 tests and subsequently culled (class 4).

We assume probabilities p1 = p(NR) and p2 = p(IR | not NR) such that

$$\log[-\log(1 - p1)] = a1 + (\text{model terms}),$$

$$\log[-\log(1 - p2)] = a2 + (\text{model terms}).$$

Model terms, common to the 2 probabilities and to the 3 tests, were herd breakdown, lactation group, interaction between herd breakdown and lactation group, and month of test. Age and milk yield were included in the model as orthogonal polynomials of degree 2. A random genetic effect was fitted for each animal and a random cow permanent environmental effect was included. The latter allows for a nongenetic covariance between observations on the same cow. The intercepts a1 and a2 are allowed to vary from test to test. This is an example of a continuation ratio model (Agresti, 2002), in this case modeled with a complementary log-log link function. The continuation ratio model was applied to the full data set (68,497 cows).

Herd Test-Date Model. The models described above do not recognize the full testing sequence; for example, an NR cow in class 1 of Table 1 is treated as if it were the outcome of a single test, but such a cow may only emerge after a repeated sequence of tests during the breakdown. Because the herd is closed from the start of the breakdown, it is possible to infer that, for example, a cow that is not present in the VetNet database would have tested NR at every skin test carried out during the breakdown. Therefore, an extended data set was developed:

(i) Animals present at the start of the herd breakdown were always present at each of the subsequent tests unless culled as an R or a 3-fold IR. If deemed present, a record was generated.
(ii) Unless indicated otherwise by the VetNet database, all cows present at a given test-date were assumed to have an NR outcome.

As before, the trait was a binary variable with 0 = NR, otherwise 1, and effects were estimated on the liability scale using a complementary log-log function. The main contemporary grouping was herd test-date, and age at test in days was included in the model as a quadratic polynomial. Cow genetic and cow permanent environmental effects were included as random variables. Given that test data over a maximum of 2 yr had been generated, neither lactation number nor milk yield was available. Note also that, depending on testing regimen, some young stock of <12 mo of age are not routinely tested. To avoid generating clear tests for animals that were not actually tested, no data was generated for cows <12 mo old.

RESULTS

Table 1 gives the number and percentage of observations for each of the 7 classes. To simplify the presentation of results where covariate adjustment has been used, an average cow was defined as a cow that was 1,600 d old, or yielded 25 kg of milk/d, or both.

Ultimate Fate Models C and D

Results from models C and D were similar. All effects in both models removed a significant amount of variation. From model C, the probability of an average cow being culled as an R or a multiple IR was highest in June and July and lowest in November and December. The difference in culling between summer and winter months was statistically significant ($P < 0.05$). The solutions for month of test for model D differed slightly from those for model C in that the probability of culling was highest in February and lowest in October. Figure 1 shows that the probability of an average cow (1,600 d old) being culled as an R decreases as her daily milk yield increases, from 0.086 to 0.042 (model C) and from 0.072 to 0.025 (model D). Figure 2 shows how the probability of being culled varies depending on the age of the cow. The probability increases from first calving to around 6 yr of age, and then decreases. Note, though, that the standard errors are increasing over this later range and consequently, results are less reliable. Heritability of liability was estimated to be 0.16 ± 0.02 from model C and 0.18 ± 0.04 from model D (Table 2), the smaller data set leading to a loss in precision; nevertheless, for both models, the evidence for genetic variation was highly significant ($P < 0.001$).

Results from the bivariate analysis of ultimate fate and daily milk yield of cows with SICCT results subsequently confirmed using gross pathology or bacteriology is given in Table 3. Heritability of liability is, as expected, consistent with the univariate analysis (model D). Heritability of milk yield is lower than the figure used in national evaluations (approximately 0.50) possibly because of the different methods of estimation (simple linear model versus a random regression test-day model). Both phenotypic and genetic correlations were negative, indicating that both management and genetics contribute to higher yielding cows being less likely to be culled as reactors.

Continuation Ratio Model

All effects in this model removed a significant amount of variation in the trait. Table 4 gives the probability of a clear result (NR), the probability of an inconclusive result, and the probability of being classed a reactor at each of the 3 possible tests, for a typical first-, third-, and fifth-lactation cow. The results show clearly that the probability NR decreases as an animal ages from first to fifth lactation, whereas the probability of being an R increases with age. The heritability of liability

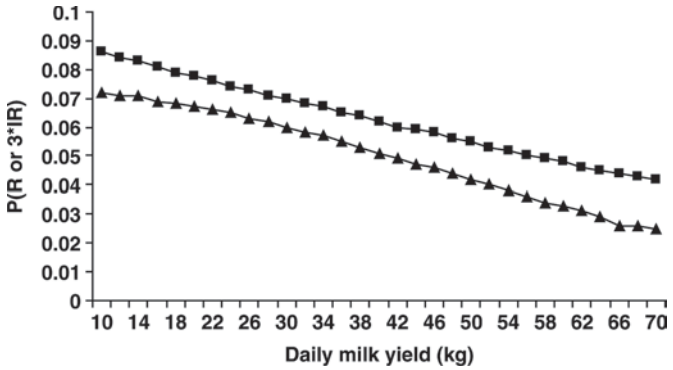

Figure 1. The effect of daily milk yield at the start of the breakdown on the risk of being culled for bovine tuberculosis over the course of the breakdown [P(R or 3 × IR)]. Squares are the results from model C (includes data on all animals), and triangles are the results from model D (data from confirmed cases only). R = reactor; IR = inconclusive reactor.

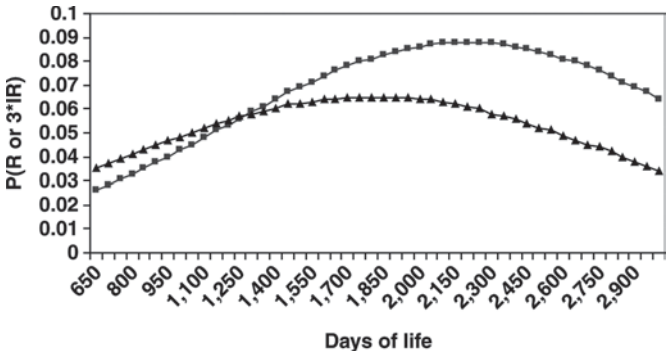

Figure 2. The effect of age from 2 to 8 yr of age at the start of the breakdown on the risk of being culled for bovine tuberculosis over the course of the breakdown [P(R or 3 × IR)]. Squares are the results from model C (includes data on all animals) and triangles are the results from model D (data from confirmed cases only). R = reactor; IR = inconclusive reactor.

from this model was estimated to be 0.08 ± 0.01 ($P < 0.001$) as shown in Table 2. Note that for these models, the heritability is for the outcome of a single stage and not the outcome of the process, which will contribute to the lower heritabilities involved.

Herd Test-Date Model

As in the other models, days of life removed a significant amount of variation in the trait. The genetic variance component and the cow permanent environmental component were both significantly greater than zero. Heritability of liability was estimated at 0.07 ± 0.01 (Table 2) and was statistically significant ($P < 0.001$). As with the continuation ratio model, the heritability was lower than that in the ultimate fate models because the heritability is for a single test classification and not for a whole process of diagnosis. The total variation attributable to an individual is the sum of heritability (h^2) and permanent environment (c^2) variation, which, as shown in Table 2, is approximately 0.4; this may appear low given that about 85% of cows test clear on all occasions. However, these observations may be reconciled by noting that the fraction relates to the underlying liability scale.

DISCUSSION

Findings from this study show that there is heritable variation in a trait defined by whether a cow is culled by the end of the breakdown. This remains true when the outcome is defined more stringently in requiring "diseased" animals to be confirmed by gross pathology or bacteriology. The genetic correlation between milk yield and the liability of being culled and confirmed with BTB suggests that selection for milk yield has not contributed to the current epidemic and that selection for BTB resistance would not conflict with improving yields of milk. On the contrary, the negative genetic correlation between milk yield and susceptibility to BTB implies that animals that are of high genetic merit for milk yield are less likely to be susceptible to BTB. Preferential treatment of high-genetic-merit animals may contribute to this association.

Heritability estimates for disease incidence traits in cattle are generally low. For example, using binary disease records on more than 450,000 Danish Holstein cows, heritabilities were obtained on the underlying scale of 0.094 for mastitis and 0.049 for other (reproductive, digestive, and feet and leg) diseases (Hansen et al., 2002). Many countries routinely evaluate and publish breed-

Table 2. Estimates of heritability (h^2) and permanent environment (c^2) for ultimate fate models C (includes data on all animals) and D (data on confirmed cases only), continuation ratio model (CRM), and herd test-date model (HTD)[1]

	Model			
Item	C	D	CRM	HTD
h^2	0.163 (0.019)	0.181 (0.044)	0.077 (0.012)	0.073 (0.013)
c^2	NA	NA	0.159 (0.013)	0.329 (0.013)

[1]Standard errors are in parentheses; NA indicates that the parameter was not part of the model.

Table 3. Genetic (above diagonal) and phenotypic (below diagonal) correlations between the risk of being culled as a reactor (u_D) and daily milk production[1]

Item	u_D	Milk production
u_D	0.19 (0.042)	−0.48 (0.13)
Milk production	−0.068 (0.028)	0.17 (0.019)

[1]Heritabilities are on the diagonal; standard errors are in parentheses.

ing values for lifespan (average heritability = 0.10) and calving ease (average heritability = 0.05; www-interbull.slu.se/national_ges_info2/framesida-ges.htm). In this analysis, estimates of heritability varied from 0.07 to 0.18, depending on the model, indicating higher genetic variance as a proportion of total variance for BTB compared with many other disease traits. Bermingham et al. (2009) also estimated significant genetic variability for susceptibility to BTB within the Irish herd, and Gonda et al. (2006), in an analysis of Johne's disease (caused by *Mycobacterium avium* ssp. *paratuberculosis*) in US Holsteins, obtained heritability estimates ranging from 0.10 to 0.16. Note, however, that their estimates had low precision mostly because of poor genetic connections between sire families. It therefore appears feasible to predict and publish breeding values for liability to BTB for dairy cows and bulls, allowing farmers and breeding companies to select breeding stock that will produce offspring with maximum resistance to BTB.

Several issues may interfere with the estimation of heritability, but in each case theoretical analysis leads to the conclusion that the effect will be to underestimate the true heritability, and the true heritability for BTB susceptibility will be greater than has been estimated here. These issues will be considered in turn:

(i) Exposure to the pathogen may be unequal among the cattle in the herd, or at least cannot be assured to be equal, and this will influence the heritability observed. However, unequal exposure is one of several systematic nongenetic factors that, given data availability, we would have included in the models as fixed effects. The fact that these effects are not included means that measured heritability is less than it would be with the more detailed model.

(ii) The measure of BTB susceptibility used is based on an imperfect diagnostic test. Some nondiseased animals are culled (i.e., imperfect specificity of the SICCT) and some diseased animals are not culled (imperfect sensitivity). Consider a test that is not specific and assume that it is perfectly sensitive: a consequence will be that the expression of the true genetic variation will be diluted by more cows from more resistant families being counted among the "diseased." A second consequence will be that the observed prevalence will increase because of these additional cows being counted as diseased. This has a 2-fold impact: variation is reduced and the conversion factors used to map the observed incidence to the scale of liability is reduced because of the increased prevalence. Therefore, the estimate of heritability on the liability scale will be severely underestimated. In contrast, consider the case in which the test is specific, in that all animals with a positive test are infected, but the test may not be perfectly sensitive. Again the genetic variation is reduced because the susceptible families are observed as being less extreme than they are. Furthermore, the observed prevalence is reduced but the conversion from the observed to liability scale is greater than it should be, counteracting in part the reduced genetic variance. Theoretical exploration (results not shown) also shows that

Table 4. For 3 categories of cows, the probabilities (P) of a nonreactor P(NR), an inconclusive reactor P(IR), and a reactor P(R) at each of 3 tests

Item	P(NR)	P(IR)	P(R)
Lactation-1 animal, 800 d old, producing 25 kg of milk/d			
Test 1	0.915	0.073	0.012
Test 2	0.898	0.096	0.006
Test 3	0.938	0.057	0.005
Lactation-3 animal, 1,600 d old, producing 25 kg of milk/d			
Test 1	0.826	0.131	0.043
Test 2	0.804	0.173	0.023
Test 3	0.877	0.102	0.021
Lactation-5 animal, 2,400 d old, producing 25 kg of milk/d			
Test 1	0.806	0.141	0.053
Test 2	0.781	0.191	0.028
Test 3	0.862	0.112	0.026

the heritability estimate will be an underestimate, but not to such a severe degree as when the test is sensitive, not specific.

The above considerations lead to the conclusion that heritability presented in this study is an underestimate of the heritability for BTB susceptibility.

A further important issue is whether these results are related to susceptibility to TB or whether they are related to the response of infected animals to the skin test (i.e., the genetic variation observed lies in the interaction between infected animals and the pathogen). This is an important issue for disease control because if the observed heritability is related to this interaction, selecting upon this genetic variation will erode the sensitivity of the test rather than reduce the prevalence of the disease. However, this too can be very largely discounted, by observing that infected cows that are not responding to the test will appear in the surviving pool of animals along with all animals that are not infected, and in the data sets analyzed here, this represents the majority of animals within herds. Therefore, if the observed genetic variation were explained by this interaction, and not by susceptibility to the disease, the genetic pattern of covariance observed would have been overwhelmingly diluted by those that are not infected. In this case, to have observed such high estimates of heritabilities would require the sensitivity of the overall testing process (not only a single skin test) to be much less than 0.5, even in the unlikely event that the true heritability for this interaction were 1. Therefore, it is reasonable to conclude that what has been observed is genetic variation in TB susceptibility.

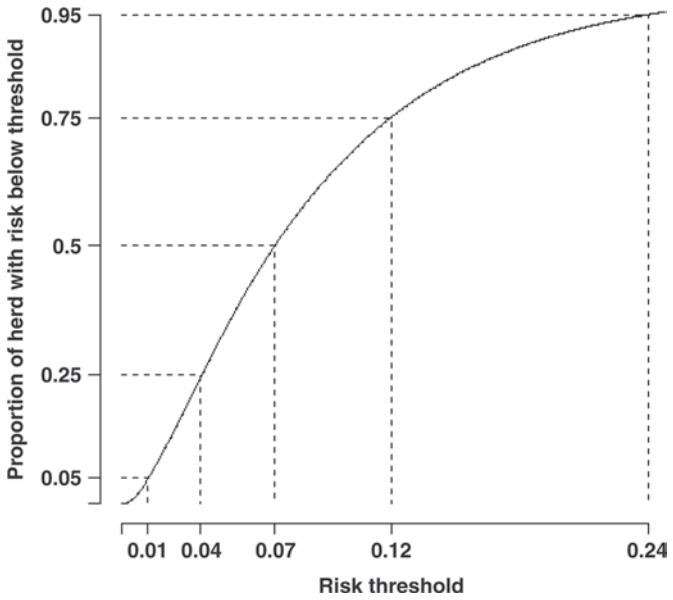

Figure 3. Cumulative distribution of individual risk in a herd with median risk of 0.07; x-axis = risk threshold, y-axis = proportion of animals in the herd with risk below the threshold.

The genetic analysis was confined to herds that had no recorded breakdown in the 4 yr before the breakdown included in the analysis where at least 2 reactors were recorded. The herds in the genetic analysis are therefore likely to be most representative of the dairy herds in GB from low incidence and low prevalence areas, which will be under 4-yearly TB testing (as opposed to annual, bi-annual or tri-annual testing). The incidence of confirmed breakdowns in GB herds in 2006 was 0.3% in areas subject to 4-yearly testing compared with 7.5% in areas subject to annual testing (Veterinary Laboratory Agency annual surveillance report for 2007; unpublished). Confirmed breakdowns comprised 47 and 69% of all incident breakdowns in 4-yearly and annual testing areas, respectively. Future work plans include the analysis of repeated breakdowns in herds to confirm the separate existence of genetic variation in resistance to TB in previously exposed animals and to test the hypothesis that this can be regarded as the same trait variation as the genetic variation observed in naïve animals.

The approach conducted throughout this analysis has been to apply linear mixed models to threshold data. Throughout the development of the theory on such models, there has been a concern on the extent of bias in the resulting estimates from the nonlinearity of the link functions between the observed 0/1 scale and the liability scale. To protect against this, the key analyses on u_C and u_D presented here have been analyzed using a variety of approaches: the results were analyzed on the observed 0/1 scale and estimates scaled using an approximate method (Robertson and Lerner, 1949); sire models have been fitted rather than full pedigree, which have the benefit of fitting smaller variances on the liability scale and hence are expected to be less exposed to bias through nonlinearity, although at a cost of loss of some of the genetic variation; simulation studies in which dummy data was generated with known parameters were analyzed to assess the potential degree of bias. The conclusion from this extensive testing is that the results are robust in this respect.

This study has shown that within a herd there is heritable variation in individual risk for susceptibility to BTB. An illustration of the extent of this is shown in Figure 3, using values derived from a normal approximation to the models fitted. The graph demonstrates that at a herd prevalence of 7% (i.e., 7% of the herd is culled during the breakdown, the average value found here), individuals within the herd have differing probabilities of being culled because of their genotype. Five percent of the cows in the herd have a probability of

being culled of <0.01, whereas 5% of the cows have a probability >0.24. The interquartile range of probabilities is between 0.04 and 0.12.

It is clear that genetic information could play an important role in control strategies for BTB in reducing the incidence of herd breakdowns and in reducing the persistence of herd breakdowns given their occurrence. It is not anticipated nor even suggested (because it is assumed that the variation is not complete resistance) that it be the sole strategy. It is clear that with a disease such as BTB, several coordinated strategies need to be undertaken, and genetics has the potential to make a substantial contribution as part of this wider effort. Irrespective of the positive use of the genetic variation for control strategies, other strategies may benefit from examining their genetic implications, because individual risk can vary widely, as shown in Figure 3.

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