Variance Components and Genome Wide Association Analysis of *Mycobacterium bovis* Infection in Dairy and Beef Cattle

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ABSTRACT: Infection of livestock with bovine tuberculosis (bTB; *M. bovis*) is of major economical concern in many countries; approximately 15,000 to 20,000 cattle are infected *per annum* in Ireland. The objective of this study was to quantify the genetic variation for bTB susceptibility in Irish dairy and beef cows. A total of 105,914 cow, 56,904 heifer and 21,872 steer single intra-dermal comparative tuberculin test records were available. Variance components for bTB were estimated using animal linear mixed models and co-variances were estimated using sire linear mixed models. The heritability for susceptibility to bTB ranged from 0.08 (heifers in dairy herds) to 0.19 (heifers in beef herds). Using 842 high density bull genotypes (n=630,352) and deregressed estimated breeding values for bTB susceptibility a genome wide association scan for bTB susceptibility was performed using a single SNP regression approach.
Keywords: Genetics; Dairy cattle; Tuberculosis; GWAS

Introduction

Bovine tuberculosis is a chronic respiratory disease caused by mycobacterium bovis. Infection of livestock with bovine tuberculosis (bTB; *M. bovis*) is of major economical concern in many countries including Ireland and the UK. Despite a long standing bTB eradication program in Ireland, progress towards eradicating the disease has been slow with the number of infected animals per year dropping from over 30,000 to approximately 15,000 since the mid 1960's.

Genetic selection for bTB resistance could be incorporated into current breeding strategies in Ireland to enhance the national eradication strategy. This approach however requires information on the extent of genetic variation for bTB susceptibility within the Irish cattle herd. Heritability for susceptibility to *M. bovis* infection has previously been documented to be 0.18 in both Irish (Bermingham et al. (2009)) and UK dairy herds (Brotherstone et al. (2010)). However, these studies were confined to dairy cattle and, to date no study has been undertaken on beef cattle.

Only one genome wide association study exists in the literature for bTB (Finlay et al. (2012)). This study however was limited in sample size (n=307), number of genomic markers (n=44,426), and reliability of the estimated breeding values of the bulls. Finlay et al. (2012) identified one genomic region on BTA 22 putatively associated with bTB susceptibility; this genomic region contained a transporter gene SLC6A6, which is known to have a function in the immune responses.

The objective of this study was to quantify the potential to genetically select for bTB in Ireland by estimating the necessary variance components for bTB using Irish field data in dairy and beef cattle. An additional objective of the present study was to identify regions of the bovine genome associated with bTB susceptibility in Irish dairy and beef cattle and elucidate the likely candidate genes and pathways contributing to these putative QTLs.

Materials and Methods

Data collection and editing. Field surveillance for bTB is performed annually in all Irish herds through the routine use of the single intra-dermal comparative tuberculin test (SICTT). The outcome of the SICTT is determined by the relative difference in the thickness of skin-folds in reaction to the bovine and avian tuberculin. Using so-called standard interpretation, if the relative bovine-avian difference is greater than 4 mm, the animal is considered a 'standard reactor'. In the present study, an 'episode' refers to the full period of herd restriction triggered by disclosure of bTB infection within a herd. Episodes were only included in the analysis if at least two standard reactors were detected from field surveillance, of which at least one of the reactors had to be home-born. Episodes with ≥10 reactors were only included if at least 20% of these reactors presented with bTB lesions postmortem, from abattoir testing. Animal records were only retained if they had moved into a herd >6 weeks prior to the start of an episode. Additionally only animals with a known sire were retained. Animals were categorized as cows, heifers or steers, based on their sex, age and parity; male animals >36 months old were discarded. After editing a total of 105,914 cow, 56,904 heifer and 21,872 steer SICTT results from dairy and beef herds were available.

Variance components. Variance components were estimated in several subpopulations including all animals, within individual animal type (cow, heifer or steer) separately, using animal linear mixed models; co-variances between animal types were estimated using sire linear mixed models. Fixed effects considered for inclusion in all models were episode, and both heterosis and recombination loss coefficients of the animal. Parity and stage of lactation were included as fixed effects in the analysis of cows. Age was included as a continuous variable in the analysis of steers and heifers. Animal gender was included in models for the combined analysis of cows, heifers and steers. Animal was included in all models as a random effect.

Sire estimated breeding values were generated from the univariate animal model for bTB susceptibility that included only cow records. The mean estimated breeding value (EBV) per breed was obtained for 1) all sires with daughter records for bTB in the data and 2) all sires with at least 10 daughter records for bTB in the data.

Genome wide association analysis. Sire estimated breeding values generated from the univariate animal model for bTB susceptibility that included only cow records, were used as a quantitative trait in a genome wide association scan for bTB susceptibility. Estimated breeding values for sires from the animal model were deregressed.

Illumina high density SNP genotypes were available on 770 Holstein-Friesian bulls. Following all edits, 630,352 autosomal SNPs remained for inclusion in the analysis. Missing genotypes were imputed using Beagle (Browning and Browning, (2007, 2009)).

Illumina Bovine50 beadchip genotypes were available on 5313 Holstein-Friesian dairy bulls; 639 of these animals also had high density genotypes. Animal genotypes were imputed to high density for each chromosome separately in Beagle (Browning and Browning (2009); Browning and Browning (2007)). A total of 842 Holstein-Friesian bulls with high density genotypes had EBVs for bTB susceptibility with reliabilities greater than 30%.

Mixed model methodology was used to estimate SNP effects for bTB susceptibility in WOMBAT (Meyer and Tier (2012)). Weighted deregressed EBVs were included as the dependent variable and animal (i.e., bull) was included as a random effect with relationships among animals accounted for. Each SNP was included separately in the model as fixed effects. Significance levels for each SNP were calculated from the resulting t-statistic assuming a two tailed t-test. To adjust for multiple testing, q-values were calculated from each p-value, using a false discovery rate of 5%.

Quantitative trait locus (QTL) regions were defined using local LD structure surrounding each SNP with a genome wide significance level of P<0.0001. Pair wise LD between SNPs was calculated using the r^2 function in PLINK (Purcell et al., 2007). A 5Mb window upstream and downstream of each SNP was used for these calculations and an r^2 threshold of 0.5 was applied. Overlapping QTL regions were combined into a single regions using the reduce function in the "GenomicRanges" (Aboyoun (2010)) R package. Genes within each of these regions were investigated, to ascertain any association with bTB susceptibility. The gene lists generated from the GWAS was then analyzed to determine which pathways were statistically overrepresented in the list. This analysis was performed using the hypergeometric algorithm and the Benjamini Hochberg correction for multiple hypothesis testing (Benjamini & Hochberg (1995)) and returned statistically overrepresented pathways.

Results and discussion.

Variance component analysis. The heritability for susceptibility to bTB in the entire dataset was 0.11 and among the sub-populations investigated ranged from 0.08 (heifers in dairy herds) to 0.19 (heifers in beef herds). The heritability estimates for bTB susceptibility in the present study are consistent with previous estimates of bTB susceptibility in both Irish dairy (Bermingham et al. (2009)) and UK dairy (Brotherstone et al., 2010) cows.

Clear variation in bTB prevalence amongst sire daughters was evident (Figure 1) with the prevalence among sire daughters ranging from 0.0 to 0.96 also clear breed differences in susceptibility to bTB were evident with Holstein-Friesian sires having a lower average EBV for susceptibility to bTB than beef breeds.

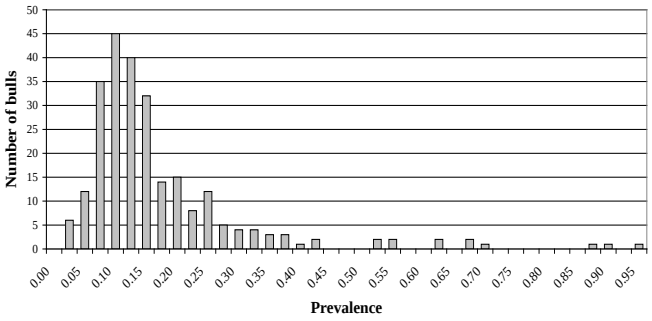

Fig 1. Distribution of mean bTB prevalence in daughters of sires with ≥50 daughters from ≥10 different herds. A wide variation in mean bTB prevalence in daughters of sires was observed.

Genome wide association analysis. A Manhattan plot representing the association between each SNP and bTB is in Figure 2. A total of 612 SNPs had a genome wide significance level, after correction for multiple testing using q-values, of P<0.0001. A total of 180 QTL regions were defined from pairwise LD between these SNPs. Within these LD blocks 15 genes were identified. The SNP with the strongest P-value (P<0.000001, BovineHD1400020824) was located on BTA14 (base pair position, 74,190,131). This SNP was however not located within any LD block. The SNP with the strongest P-value located within an LD block was BovineHD0100029059 (BTA1, base pair position 101,899,126). The block containing this SNP was 180kb in length, one gene was located within this QTL region, SERPINI2. A 27kb QTL region on BTA10 contained 10 genes (AGGF1, SV2C, GRM7, CRBN, IZUMO3, TRNT1, PPARD, GIMAP7, LHFPL2 and SCAMP1). Two genes were located within a 29kb QTL region on BTA19 (GIMAP7 and SPATA20). The final QTL region (74kb) which contained genes was located on BTA23. This QTL region contained one gene DEF6. The gene

CRBN is a component of some E3 protein ligase complexes, which mediate proteasomal degradation. Proteasomal degradation plays a crucial role in the function of the adaptive immune system, initiating the expression of MHC class 1 proteins on the surface of infected cells. A mutation of this gene could affect the host susceptibility to bTB infection. Since mycobacterium bovis replicates within macrophages, preventing the expression of MHC class 1 proteins on the surface of macrophages would prevent apoptosis of the macrophages and allow the bacterium to replicate more within host macrophages. The gene DEF6 is known to have a function in T helper cell development and activation and is a gene expressed broadly throughout the immune system and can be detected in both T and B cells. A mutation in DEF6 could greatly increase host susceptibility to bTB infection, as T cell would have defective development and hence the host would have a reduced immune response to infection.

Conclusion

Because bTB is of economic importance and phenotypic information is available through the routine testing of all animals, the existence of genetic variation in bTB observed in this study suggests that bTB susceptibility should be included in a national breeding program.

Identifying genes underlying host susceptibility to bTB infection could enhance current development toward vaccinations or treatment of bTB in either the hosts or within wild life reservoirs.

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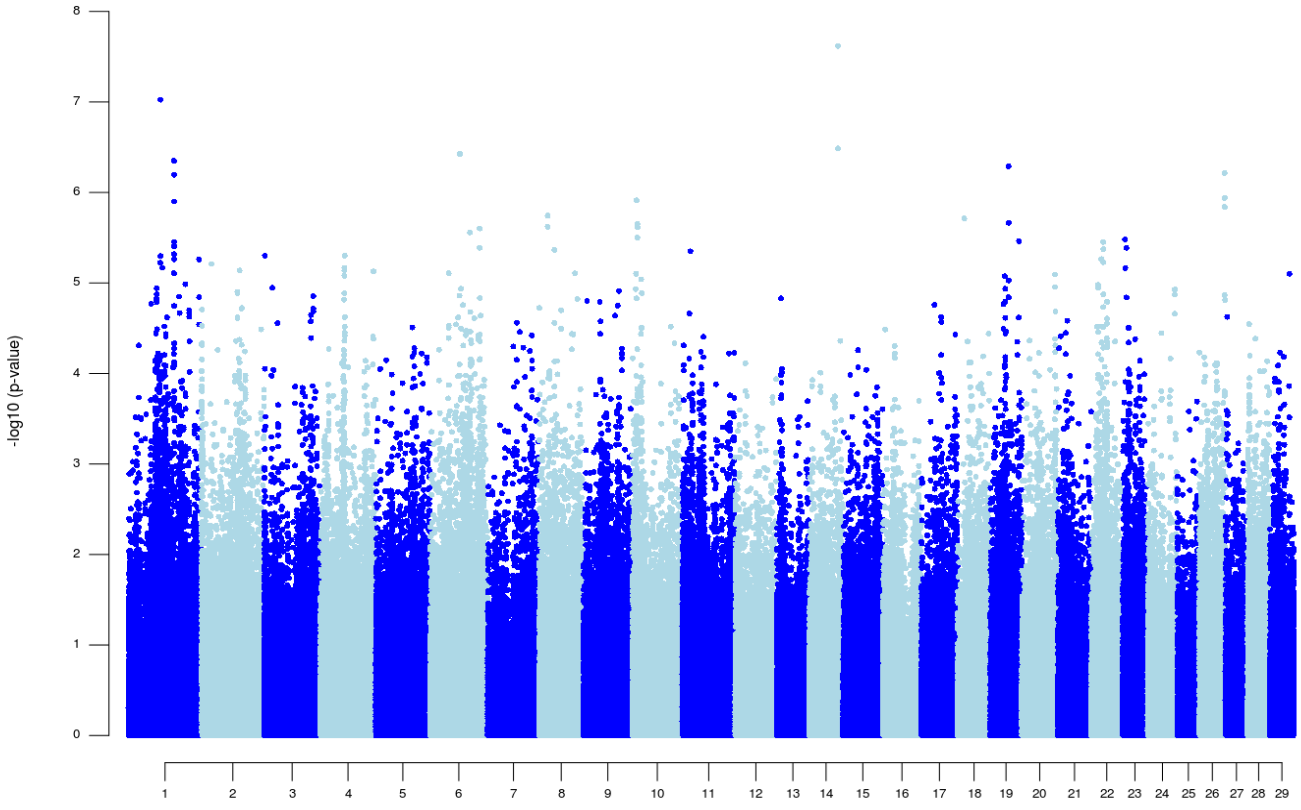

Fig 2. Manhattan plot displaying results (-log10 of p-values) of genome wide scan with respect to genomic position, for bTB susceptibility in dairy and beef sires. Analysis was performed using a single SNP regression approach.