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Predicting Susceptibiliy to Johne's Disease in New Zealand Dairy Cattle

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ABSTRACT: Johne's disease (JD) is a chronic, inflammatory gastrointestinal disease caused by Mycobacterium avium ssp. Paratuberculosis. JD has a significant economic impact on the New Zealand (NZ) dairy industry. The objective of this study was to develop a predictive genomic test for susceptibility to JD in NZ dairy cattle. Cows were confirmed as being JD affected (JD+) by ELISA of milk and serum samples. 1,833 JD+ cows were genotyped using the Illumina SNP770 Bead Chip. 6,849 Control cow genotypes (50K) representing the general population were chosen to match the breed proportion profile of the JD+ cows and their genotypes were imputed to 770K for analysis. The genomic merit for susceptibility to JD of 1 Mb windows across the genome was estimated using a Bayes B model. Test accuracy was evaluated using 10-fold cross-validation. Results suggest that a genomic test for JD susceptibility with prediction accuracy at a level suitable for practical use is possible.

Keywords: dairy cattle; Johne's Disease; susceptibility; genomics

Introduction

Johne's disease (JD) is a chronic, inflammatory gastrointestinal disease, particularly affecting cattle, sheep and deer (Purdie et al. (2011)). Johne's disease is characterized by lesions in the distal part of the ileum, hindering nutrient uptake (van Hulzen et al. (2012)), resulting in chronic diarrhea, emaciation, decreased milk production, and eventually death (Gonda et al. (2006)). The causative agent of JD is Mycobacterium avium ssp. paratuberculosis (MAP) (Gonda et al. (2006); Pant et al. (2010); Purdie et al. (2011)), an infectious bacterium that can be spread through faecal shedding, and can persist in the environment for many months. Commercial vaccinations are available, although they tend to delay the onset of clinical signs rather than prevent the disease, and these vaccinations cause a false-positive reaction to tuberculosis tests.

Apparent herd prevalence has been reported to range between 7 % in Austria to 60 % in New Zealand (Grant (2005)). The presence of JD in the national herd represents a large economic loss to the dairy industry, mainly due to reduced milk production and premature culling (Ott et al. (1999)).

Previous studies have demonstrated the presence of genetic variation for susceptibility to JD (Gonda et al. (2006); Attalla et al. (2010)) and some have identified genomic regions associated with increased susceptibility to JD (Kirkpatrick et al. (2010); Minozzi et al. (2012); van Hulzen et al. (2012); Sherlock et al. (2013)). The genomic signals identified vary between these previous studies, however these are in different populations (both genetics/breed and production systems).

The objective of this study was to develop a test that could be applied to the genomic profiles of NZ dairy cattle to predict the susceptibility of an animal (and its progeny) to MAP infection.

Materials and Methods

The genomic profiles of cows identified as Johne's disease positive (JD+) from within the NZ dairy population were compared to the profiles of a Control group representing the general population to generate a predictive test for susceptibility to JD.

Johne's disease diagnosis. Diagnostic testing of milk and blood samples employed an enzyme-linked immunosorbent assay (ELISA) marketed as the IDEXX Paratuberculosis Screening Ab Test (www.idexx.com).

Herds were initially prioritised for individual cow screening by ELISA on bulk milk samples.

Subsequently, routine herd test milk samples from individual cows in these herds were tested by ELISA used to identify potential JD+ case cows. A blood plasma sample was collected from milk reactor cows to confirm the ELISA positive status. The ELISA sample to positive control optical density ratio thresholds were set at 0.4 and 0.7 for milk and plasma respectively, as per kit instructions prior to 2010. Only cows testing positive on milk as well as plasma ELISA were classified as JD+.

Genotypes. DNA for genotyping was extracted from the blood samples that were used to confirm cows as JD+. Genotyping was performed with the Illumina Bovine SNP770 Bead Chip and resulted in 1833 valid JD+ genotypes with a sample call rate of 95% or greater.

Genotypes from 23,097 cows, representing the general NZ dairy cow population, were made available to the study and formed the Control group following the approach taken by the Wellcome Trust Case Control Consortium (2007). Genotypes for Control cows were obtained using the Illumina Bovine SNP50 Bead Chip and were im-

puted to the 770,000 SNP using Beagle v3.3.2 (Browning and Browning (2009)). SNP with a minor allele frequency of less than 1%, an imputation R^2 of less than 90% in the reference, or with poor clustering characteristics were removed from the analysis. In addition, any SNP common to both the SNP50 and SNP770 Bead Chips were removed to minimize the effects of between-panel differences on the analysis. The remaining 626,033 SNP were included in subsequent analyses.

Analysis. To account for breed stratification, JD+ cows were grouped into 10 Holstein-Friesian/Jersey breed classes. Control cows from these same classes were chosen at random to generate a matched control of 6,849 cows. The total number of animals in the matched control was determined by the number of Control cows available in the limiting breed class.

A categorical Bayes B model ($\pi = 0.99$) (Meuwissen et al. (2001)) was fitted using the software GenSel v4.53R (Fernando and Garrick (2008)). Year of birth, and proportions of Jersey, Holstein-Friesian and overseas' genetics were fitted as covariates. A total of 50,000 iterations were used, with the first 5,000 excluded as the burn-in. The model estimated the genomic merit for combinations of SNP in each 1 Mb windows across the whole genome in a training population. These estimates were then used to predict the genome-wide genomic merit for susceptibility to JD in test populations.

Ten-fold cross-validation was used to determine the accuracy and robustness of the predictions from the model. Animals were randomly assigned to 1 of 10 folds. The susceptibility to JD of each of the 10 folds was predicted using the predictive model generated using the 9 remaining folds.

Results and Discussion

Figure 1 shows that the median predicted genomic merit for susceptibility to JD is significantly higher in the JD+ group than the Control group. The Control group was representative of the general population (rather than being JD negative) and so would be expected to include a number of JD positive animals. The cluster of Control animals with high genomic merit predictions is therefore un-surprising and had these animals been exposed to JD and tested, they may well have tested JD positive.

The area under the Receiver Operating Characteristic (ROC) curve (Figure 2) is equivalent to the probability that a randomly chosen positive animal will be ranked higher than a randomly chosen negative animal. The average probability of 0.90 suggests the test is a good to excellent classifier. Kirkpatrick et al. (2010) have previously predicted JD susceptibility in the US Holstein population (approximately 500 genotyped JD+ animals on the SNP50 panel) using sets of up to 100 markers and obtained an AUC of 0.73.

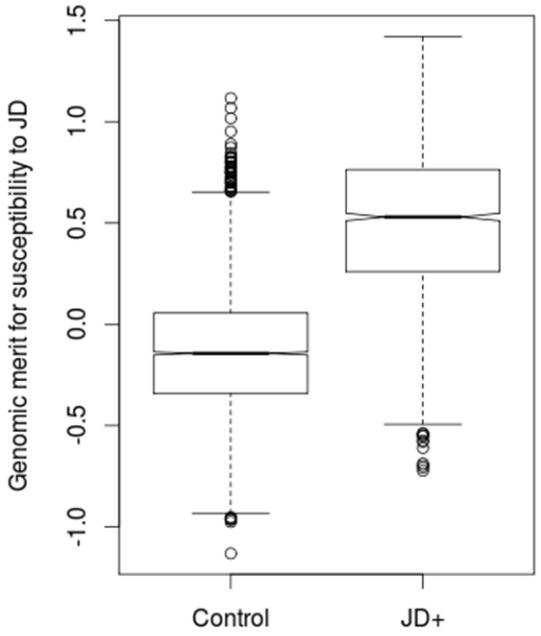

Figure 1: Genomic merit for susceptibility to Johne's Disease (JD) by JD status.

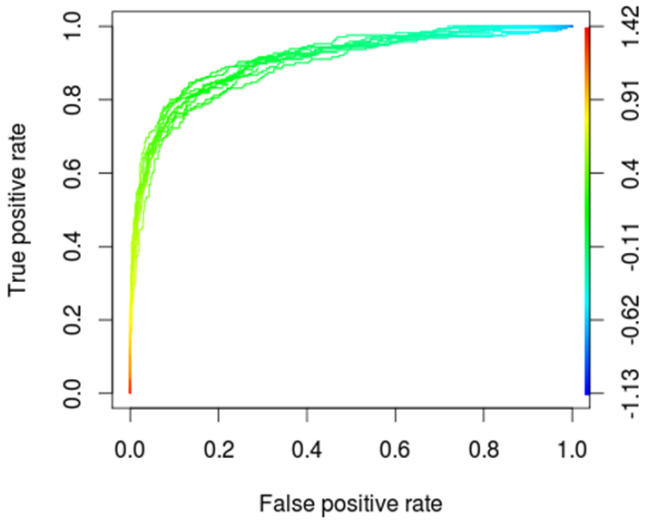

Figure 2: Receiver Operating Characteristics (ROC) curve for ten-fold cross-validation. Each line represents a model.

The predictive power of the current test is higher than would be expected for a trait with a heritability estimated at 0.23 (Back and Lopez-Villalobos, unpublished). One potential reason for this is the random allocation of animals to the folds perhaps resulting in closely related animals in both the training and test populations. This would tend to over-estimate the predictive power of the test. Further work is planned to investigate whether allocating animals to the folds in such a way as to minimize the relatedness between folds will reduce the predicted accuracy of the test.

Conclusion

The results presented suggest that it is possible to develop a test capable of predicting the susceptibility of an animal to MAP infection from its genomic profile with an accuracy that will have practical application. Additional work is required to further investigate the accuracy of the test and determine how best to integrate it into the NZ dairy industry breeding scheme.

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