METHODS

Cows identified as Johne's disease positive (JD+) from within the NZ dairy population were compared to a Control group representing the general population to identify genomic regions associated with 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 imputed 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 reduce 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 multi-SNP, genome-wide association study (GWAS) using the Bayes B method ($\pi = 0.99$) (Meuwissen *et al.* 2001) was performed 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.

RESULTS AND DISCUSSION

The GWAS detected multiple signals across the genome associated with susceptibility to Johne's disease (Figure 1).

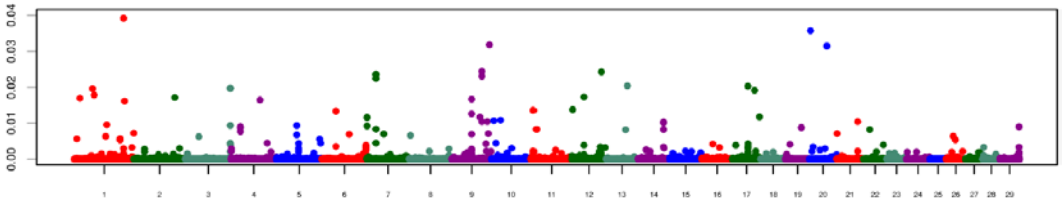

Figure 1. Bayes effect variances for an association of single nucleotide polymorphisms (SNP), with Johne's status, from a multi-SNP, Bayes B genome-wide association study

A number of the signal locations are within 1Mbp of immune-related genes. Of particular note are signals on Chromosome 3 and 7 (Figure 2) that correspond to the receptor (CSF2RA) and ligand (CSF2) for Colony Stimulating Factor 2 respectively. CSF2RA has been previously linked to response to infection by *Mycobacterium bovis* (Meade *et al.* 2008). There was no significant overlap between the major signals identified in this study and those reported in previous studies (Kirkpatrick *et al.* 2010; Minozzi *et al.* 2012; van Hulzen *et al.* 2012).

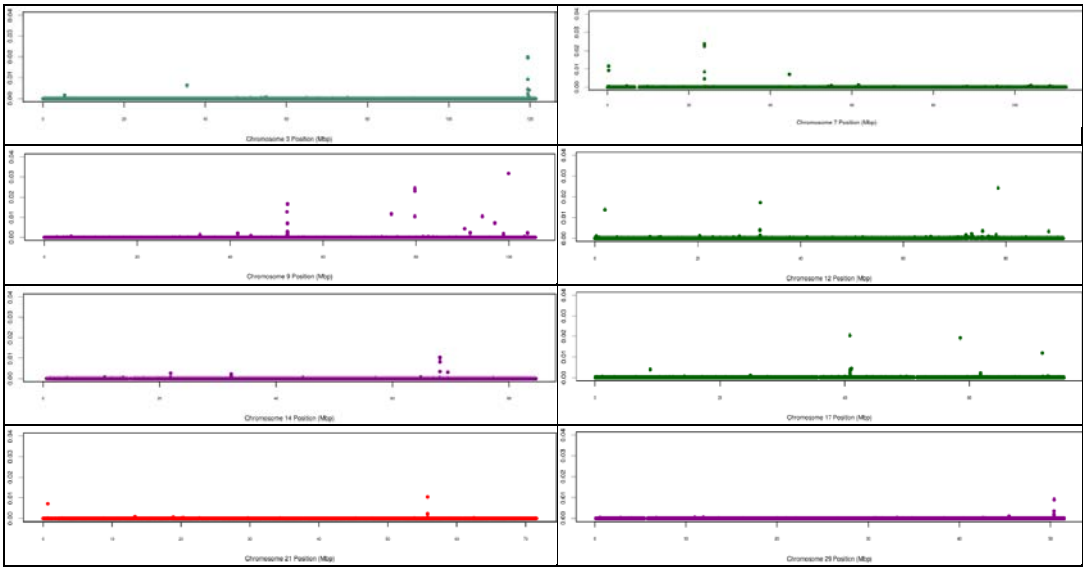

Figure 2. Bayes effect variances for an association of single nucleotide polymorphisms (SNP) with Johne's status on Chromosomes 3, 7, 9, 12, 14, 17, 21 and 29, from a multi-SNP, Bayes B analysis

That these signals tend to be associated with meaningful regions suggests that the study is targeting biologically relevant genetic structures and deeper investigation of these markers may help further illuminate the biological pathways contributing to susceptibility to Johne's disease. More immediate benefit may be gained by using the data to develop a predictive test that could be applied to an animal's genomic profile to predict the susceptibility of the animal (and its progeny) to MAP infection.

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