FURTHER EVIDENCE THAT MAJOR GENES AFFECT HOST RESISTANCE TO NEMATODE PARASITES IN COOPWORTH SHEEP

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SUMMARY

Recently, mixed inheritance model computer programs based on Markov Chain Monte Carlo methods have been developed with the ability to analyse extensive animal pedigrees. They partition the observed variation into variance due to: non genetic effects, polygenic effects, bi-allelic major locus effects and residual effects. They do not require genotype information. This technique was used to examine data from a randomly mated Coopworth sheep flock in New Zealand where faecal strongyle (FEC) and *Nematodirus* (NEM) nematode egg count information had been collected. All egg counts were initially transformed using the function $\log_e(x+50)$. The final model included the effect of sex/year contemporary group, birth day and polygenes in addition to a major, autosomal bi-allelic locus. Results from the analyses suggested a dominant autosomal gene for host susceptibility was affecting strongyle FEC values in the autumn period with estimates of 0.79 log eggs/g for the effect of a single allele, and a dominance effect of 0.96 log eggs/g. Host susceptibility for summer NEM was affected by a recessive gene with estimates of 0.64 and -0.68 respectively. The identification and characterisation of these QTL(s) may result in novel methods of internal parasite control.
Keywords: Quantitative trait loci, host resistance, sheep, nematode parasites.

INTRODUCTION

Currently, there is a large international effort underway to identify and locate polymorphic genes with alleles having a large effect on productive traits in humans and livestock. Such genes are commonly called quantitative trait loci (QTL). Progress in sheep has been enhanced by the availability of mapped highly polymorphic DNA markers (Crawford *et al.*, 1995) which make comprehensive linkage studies possible. While these linkage studies, commonly called genomic scans, are a highly effective in characterising QTL, they are not efficient when screening large populations for putative major loci. This is best achieved using purely biometrical methods. Results of these analyses can then be used in the planning of a mating design to confirm the QTL with the aid of markers. A previous study using a biometrical method based on regression has produced evidence in three separate New Zealand flocks that a major locus was segregating for strongyle faecal nematode egg count (FEC). In the one flock where it was measured, a major locus for *Nematodirus* faecal egg count (NEM), also appeared to be segregating (McEwan *et al.* 1997). While this technique was numerically efficient, it does not estimate the true level of polygenic variation nor the standard errors of the parameters. Thus it was of interest to independently verify these results using an

alternative technique, prior to generating progeny suitable for a genomic scan. This study reports results of applying a Gibbs sampling approach to the most promising data set.

METHODS

Phenotype measurements. Details have been reported in McEwan *et al.* (1997). Briefly, information recorded from a Coopworth progeny test flock based at Woodlands Research Station in Southland was used in the current study. FEC from 2 natural field challenges were recorded approximately 8 and 16 weeks after weaning at 10 weeks of age. The procedure involved the lambs being drenched at weaning and then monitored until the flock mean FEC rose to around 1000 strongyle eggs per gram of faeces (epg), at which time all individuals were individually faecal sampled and drenched. This process was then repeated. Samples were counted to the nearest 50 epg. The challenges consisted of predominantly *Ostertagia* spp. for the first challenge, although *Nematodirus* spp. was also identified and recorded separately at this time. *Trichostrongylus* spp. predominated in the second challenge.

Analysis. Initially, the results were transformed using the function $\log_e$(measurement + count unit) prior to analysis in order to normalise the variance. Fixed effects included contemporary group (year and sex) and birth day as a covariate. Based on the results of the regression analyses (McEwan *et al.*, 1997) two traits, FEC at the second challenge (FEC2) and NEM at the first challenge (NEM1) were identified as potentially segregating for major loci and these were investigated in the current analysis. Data collected from the 1990 to 1994 years were used, consisting of pedigrees of 5844 individuals with 113 sires and 1406 dams. FEC2 was recorded in 3983 individuals and NEM1 in 4054 individuals.

GIBBS sampling. Briefly, the mixed inheritance model definition is:

$$\mathbf{Y = Xb + ZQg + Zu + e}$$

where **Y** is a vector of observations, **X** is a design matrix relating observations to fixed effects, **b** is a vector of fixed effects, **Z** is a design matrix relating observations to animals, **Q** is a design matrix of size n x 2, with n being the number of animals containing the probabilities of carrying one or two copies of the putative QTL allele, **g** is a vector of QTL genotype effects, **u** is a vector of random polygenic effects for all individuals in the pedigree and **e** is a vector of residual errors. The Gibbs sampler uses a Markov chain to indirectly marginalise the joint density, with respect to all parameters of interest specified in the mixed inheritance model. A detailed description of a Gibbs sampler as applied to a mixed inheritance model has been given by Janns *et al.* (1995). The present study used a similar procedure. However some differences exist and these will be outlined. Firstly, Janss noted that a non-genetic effect was updated using solutions of the remaining non-genetic effects derived from a previous sampling state (analogous to Jacobian iteration). The approach used in the present study was to update non-genetic effects with solutions of the remaining non-genetic effects derived from the present sampling state (analogous to Gauss-Seidel iteration). Major locus genotypes and polygenic effects were updated using the neighbourhood set scheme of Janss, where the effects of parents, progeny and mates are taken as known. However sampling of new realisations for a

sire and its final progeny jointly as a block was not considered. Janss suggests that without blocking the Markov chain has a propensity to remain stuck in subspaces of the parameter space. To facilitate mixing in our implementation of the Gibbs sampler, the strategy of randomly sorting the pedigree prior to each new sampling state was adopted. Janss considered only an additive, bi-allelic locus where the allele effect was estimated as the deviation of homozygotes from an assumed mean of zero. In the present study the approach was to estimate the effect of one or two copies of the allele. Under this nomenclature dominance can be estimated. Thinning was applied in that a realisation was taken every 5 samples. The decision to use 5 as the thinning parameter was arbitrary. Prior values of parameters were those obtained from the regression analysis of the same data set (McEwan, *et al.* 1997). The number of samples was 50,000 with a burn-in period of 2500 iterations (based on 3 times the number of iterations taken for the estimates to stabilise).

Table 1: Estimates of the size, mode of inheritance and frequency of host parasite resistance QTLs in the Woodlands Coopworth progeny test flock[1]

	FEC2		NEM1	
	estimate	(SD)	estimate	(SD)
variance				
QTL	0.061	0.021	0.421	0.012
polygenic	0.071	0.014	0.028	0.009
residual	0.333	0.017	0.141	0.008
polygenic h^2	0.17	0.03	0.16	0.05
QTL effect				
one copy	1.75	0.07	-0.04	0.04
two copies	1.59	0.10	1.25	0.05
a	0.79		0.64	
d	0.96		-0.68	
allele freq.	0.86	0.03	0.75	0.02

[1]FEC2 and NEM1 are the transformed (loge[x+50]) strongyle faecal egg count (eggs/g) at the end of the second challenge and the *Nematodirus* faecal egg count (eggs/g) at the end of the first challenge respectively. Abbreviations: SD, standard deviation of the estimate; a, additive allele effect; d, dominance effect estimated from the effects of one and two copies of the QTL.

RESULTS AND DISCUSSION

The results of using the Gibbs sampling mixed inheritance model program are shown in Table 1. The results support those obtained by the regression approach, but also provide additional information. In particular, based on approximate significance levels calculated from the SD of the QTL variance estimates it would appear likely that a QTL is segregating in this flock for both FEC2 ($P<0.01$) and NEM1 ($P<0.001$). In the earlier report, heritability had been fixed at 0.1 and these results suggest that this was an underestimate of the true level of polygenic variability. For FEC2, the QTL accounted for 0.15 of the sum of QTL and residual variances.

The corresponding estimate for NEM1 was 0.75. The regression approach had previously estimated values of 0.36 and 0.70. The estimates of allele frequency of the QTLs in the current analysis were similar for both traits at 0.86 for FEC2 and 0.75 for NEM1, somewhat higher than the earlier regression estimates of 0.69 and 0.60. The estimated effect of QTL alleles were similar to the earlier report. In the case of FEC2 it would appear that the inheritance is slightly over-dominant for susceptibility to infection. The situation was reversed for NEM1 as it was completely recessive for susceptibility to infection.

The current work cannot positively identify whether the same QTL identified for FEC2 is also that affecting NEM1, which requires linkage analysis. Breeding of this flock is now concentrating on increasing the frequency of the recessive FEC2 allele so that large half sib pedigrees segregating for the allele can be generated and a genomic scan undertaken in order to independently validate these observations. Hopefully it will also identify closely linked polymorphic markers which allow marker assisted selection to be undertaken (Meuwissen and Goddard. 1996) in this difficult to measure trait. Previous reports have suggested that QTLs for host resistance to nematode parasites may exist in sheep with Stear *et al.* (1996) finding in Scottish Blackface lambs, a significant association between alleles in the MHC region located on chromosome 20 and FEC. In a separate study Gulland *et al.* (1993), reported an association between FEC and the ADA locus, located on chromosome 13.

In summary, the results from the mixed inheritance model based on Gibbs sampling techniques were almost identical to those previously reported using the regression approach. Both techniques identified a dominant QTL affecting susceptibility to FEC2 and a recessive gene affecting susceptibility to NEM1. While considerable caution still needs to be exercised until results from genotyping studies unambiguously identify the genomic region involved, these methods show considerable potential to aid identification of individuals and flocks segregating for economic QTLs and will aid in mating designs for subsequent locus identification.

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