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Identification of quantitative trait loci affecting resistance to gastrointestinal parasites in a double backcross population of Red Maasai and Dorper sheep

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Summary

A genome-wide scan for quantitative trait loci (QTL) affecting gastrointestinal nematode resistance in sheep was completed using a double backcross population derived from Red Maasai and Dorper ewes bred to F_1 rams. This design provided an opportunity to map potentially unique genetic variation associated with a parasite-tolerant breed like Red Maasai, a breed developed to survive East African grazing conditions. Parasite indicator phenotypes (blood packed cell volume – PCV and faecal egg count – FEC) were collected on a weekly basis from 1064 lambs during a single 3-month post-weaning grazing challenge on infected pastures. The averages of last measurements for FEC (AVFEC) and PCV (AVPCV), along with decline in PCV from challenge start to end (PCVD), were used to select lambs ($N = 371$) for genotyping that represented the tails (10% threshold) of the phenotypic distributions. Marker genotypes for 172 microsatellite loci covering 25 of 26 autosomes (1560.7 cM) were scored and corrected by GENOPROB prior to QXPAK analysis that included Box–Cox transformed AVFEC and arcsine transformed PCV statistics. Significant QTL for AVFEC and AVPCV were detected on four chromosomes, and this included a novel AVFEC QTL on chromosome 6 that would have remained undetected without Box–Cox transformation methods. The most significant *P*-values for AVFEC, AVPCV and PCVD overlapped the same marker interval on chromosome 22, suggesting the potential for a single causative mutation, which remains unknown. In all cases, the favourable QTL allele was always contributed from Red Maasai, providing support for the idea that future marker-assisted selection for genetic improvement of production in East Africa will rely on markers in linkage disequilibrium with these QTL.

Keywords East Africa, gastrointestinal parasites, marker-assisted selection, quantitative trait loci, sheep.

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Introduction

Gastrointestinal (GI) nematode parasites are one of the most important disease constraints limiting small ruminant productivity in both tropical and temperate regions of the world (Over *et al.* 1992; Perry *et al.* 2002). Reliance on

anthelmintic treatment as a method for control has imposed strong selection pressure, resulting in the emergence of anthelmintic resistant strains of parasites (Waller 1997; Besier 2007). In addition, excessive drug usage is under increasing scrutiny because of an increased awareness of environmental issues, while in tropical developing countries, anthelmintics are often unavailable or too costly for subsistence farming. For these reasons, alternative control strategies are being sought that lessen the dependence on anthelmintics and reduce the cost of parasite control, yet allow an increase in production under challenging grazing conditions.

One potential alternative means of controlling gastrointestinal parasites is the breeding of genetically resistant livestock (Woolaston & Baker 1996; Sonstegard & Gasbarre 2001). Research has shown that Red Maasai sheep native to East Africa are genetically resistant or resilient to GI nematode parasites, particularly *Haemonchus contortus,* while Dorper sheep are relatively more susceptible (Preston & Allonby 1978, 1979; Mugambi *et al.* 1996, 1997; Wanyangu *et al.* 1997; Baker *et al.* 2003).

In 1997, a breeding programme was initiated to create a resource population for mapping regions of the ovine genome that control resistance to GI parasites predominant to regions of Africa that currently rely on production of Red Maasai sheep (Mugambi *et al.* 2005a,b). Similar research is of global interest, as quantitative trait loci (QTL) for parasite indicator traits have been identified in other sheep breeds, including the Merino in Australia (Beh *et al.* 2002; Marshall *et al.* 2009), the Romney in New Zealand (Crawford *et al.* 1997, 2006), the Scottish Blackface (Davies *et al.* 2006) and Soay (Beraldi *et al.* 2007) in Great Britain, multiple breeds (Sarda, Lacaune, Barbados Blackbelly and INRA401) in France (Moreno *et al.* 2006) and the Florida Native and Suffolk in the USA (Cockett *et al.* 2005; Hadfield *et al.* 2008, 2009). Our study is the first report of QTL detection in a double backcross population derived from native sheep adapted for production in the extreme conditions of East Africa.

Materials and methods

Mapping population and phenotypic traits

Breeding to generate the double backcross resource families was initiated in November 1997 at the Kapiti Plains ranch in the semi-arid highland region of Kenya, and lamb crop production ($N = 6$) occurred at 6-month intervals until the end of 2000 (Mugambi *et al.* 2005a). Red Maasai (R) and Dorper (D) ewes were mated with 6 F_1 rams (5 $D_{sire} \times R_{dam}$ and 1 $R_{sire} \times D_{dam}$) to produce just under 200 half-sib progeny per sire (1064 lambs in total). This mating design produced either of two crossbred genotypes, that is, ¾ D/¼ R or ¾ R/¼ D lambs. Two of the D x R rams were half sibs, and the rest were unrelated.

Pedigrees and weight were recorded at birth for each lamb. The experimental protocol to generate parasite indicator phenotypes involved natural challenge with GI nematodes from grazing infected pastures (Mugambi *et al.* 2005a) immediately followed by an indoor trickle challenge with *H. contortus* (Mugambi *et al.* 2005b). Only data recorded during the grazing of infected pasture are analysed in this paper. Specifically, lambs were weaned (~3 months of age) and treated with an anthelmintic prior to relocation for a single grazing challenge on pastures at ILRI's Kabete experimental farm, where the predominant GI nematode parasites were *H. contortus* and *Trichostrongylus spp.* Live weight (LWT), faecal egg count (FEC) and blood packed cell volume (PCV) were recorded weekly.

Five traits were analysed. The average of two measurements taken 1 day apart at the end of the grazing challenge was determined for FEC (AVFEC), PCV (AVPCV) and LWT (AVLWT). In addition, the PCV at the start of the challenge period (PCVST) was analyzed, and the decline in PCV from the start to the completion of the pasture challenge (PCVD) was calculated. Analysis of variance was carried out for each trait fitting a fixed effects model, which included significant main effects ($P < 0.05$) for breed composition (¾ R, ¾ D), sire group (1–6), sex (male, female), crop (1–5), birth rank (single, multiple), age of dam (2–5+), lamb age (as a linear covariate) and any significant ($P < 0.05$) interactions among these effects. Heritability estimates for all traits analysed in this study were reported by Baker *et al.* (2003). Those related to PCV ranged from 0.12 to 0.14, and those for FEC ranged from 0.15 to 0.19.

After adjusting for significant environmental effects, the distributions of AVFEC (logarithm transformed), AVPCV and PCVD were used to identify the 10% most resistant and 10% most susceptible lambs for genotyping (Darvasi & Soller 1992). The subset of 371 lambs chosen for selective genotyping consisted of 192 resistant lambs (avg. 18.0% of lambs/sire family, range 12.9–20.8%), 173 susceptible lambs (avg. 16.3% of lambs/sire family, range 14.6–23.4%) and six lambs that were resistant for one trait but susceptible for another. A pedigree file containing 1081 animals was used for QTL detection.

Both AVFEC and the PCV traits were transformed prior to QTL detection to account for their non-normal distribution. AVFEC was analysed using a y_i^{λ} scale obtained from the Box–Cox transformation family, in which $y_i^{\lambda}=(y^{\lambda}-1)/\lambda, (\lambda \neq 0)$ or $y_i^{\lambda}=log(y_i), (\lambda=0)$. An adaptation of the algorithm proposed by Hyde (1999) was used to estimate the maximum likelihood of λ. The Box–Cox transformation was demonstrated as a FEC transformation method that is better at normalization than any simple logarithm transformation (Silva *et al.* 2007). The arcsine transformation, as suggested by Mosteller & Youtz (1961), was used to transform the traits expressed as percentages (AVPCV, PCVST and PCVD). The AVPCV, for instance, can be made nearly 'normal' if the square root of AVPCV is used with the arcsine transformation.

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Genotyping and linkage map

Genomic DNA was isolated from whole blood (Sambrook *et al.* 1989), and importation into the U.S. was done under APHIS regulations. Ovine microsatellite markers were chosen from the domestic International Mapping Flock (IMF) sheep genetic map (http://rubens.its.unimelb.edu.au/~jillm/jill.htm), and selection criteria included linkage group position (marker interval spacing and chromosome coverage), amplification fidelity and polymorphic information content (PIC) values. Forward primers were fluorescently labelled to detect amplified PCR products by fragment analysis application on ABI-310, ABI-3100 and ABI-3730XL automated sequencers (Foster City, CA). All PCRs and marker multiplex combinations were essentially done as described by Schnabel *et al.* (2005). More than 250 markers were tested for amplification conditions and multiplexing fidelity using DNA from USMARC (Clay Center, NE) Dorper Rams and also tested for heterozygosity in the F_1 rams. Marker genotypes were scored using GENEMAPPER 3.7 software from Applied Biosystems (Foster City, CA, USA). As a check of semi-automated genotype calls, initial non-inheritances were detected using CERVUS (Marshall *et al.* 1998). Marker order and relative position were determined using Cri-Map version 2.4 (Green *et al.* 1990) and compared to the IMF reference linkage map for congruence. For QTL detection, linkage group positions along the IMF sheep linkage map were used for final marker positioning, followed by checking for unlikely double recombinants using GENOPROB (Thallman *et al.* 2002). Genotype and grand-parental origin probabilities were estimated for each of the genotyped animals using all available information (genotype, map and pedigree). Individual genotypes with low probability as defined by GENOPROB (pGmx <0.95) were excluded from further analysis. In GENOPROB, this parameter is the probability that the genotype is correct.

DNA marker and QTL analyses

To quantify the genotypic information across the genome, the genotypic information coefficient (GIC) (Van Ooijen 2009) was estimated, which is analogous to the marker information content defined by Knott *et al.* (1997). Segregation distortion and PIC were calculated for the population using JoinMap (Van Ooijen & Voorips 2001) and CERVUS 2.0 software (Kalinowski *et al.* 2007), respectively.

Backcross data were analysed with standard interval mapping (Lander & Botstein 1989) using a least squares regression framework (Haley & Knott 1992; Haley *et al.* 1994) in QXPAK v.2.13 (Pérez-Enciso & Misztal 2004). The QXPAK package implements multitrait, multiQTL options and can be applied to populations of any complexity using all marker and pedigree information jointly.

In all analyses, the general model used was

$$y_i = \mu + \text{sex}_i + \text{lamb\_crop}_i + \text{birth\_type}_i + \text{dam\_age}_i + \text{dam\_breed}_i + \beta c_i + [P(g_i \equiv VV) - P(g_i \equiv WW)]a + u_i + e_i$$

where y_i is the record of individual i, β is the covariate coefficient, c is the age of the lamb, a is the QTL additive effect, u is the infinitesimal genetic effect and e is the residual. The coefficients $P(g_i \equiv VW)$ are the probabilities, obtained via Markov chain Monte Carlo algorithm, of the individual i having alleles of the breed origin V and W at the position of interest (Pérez-Enciso & Misztal 2004). The Haldane function was assumed to obtain these probabilities. The infinitesimal effect was treated as random, with covariance $\boldsymbol{A}\sigma_u^2$, $\boldsymbol{A}$ being the numerator relationship matrix.

Nominal P-values were obtained via likelihood ratio tests and the chi-squared approximation. As shown by Mercadé *et al.* (2005), a 1% chromosome-wise significant P-value corresponds roughly to a nominal P-value of 0.001, and therefore we considered that nominal P-values <0.001 were significant for QTL testing. Significant genome-wide thresholds for the QTL were calculated using the procedure described by Lander & Kruglyak (1995). For additive effects, P-values smaller than 3.38×10^{-3}, 1.69×10^{-4} and 3.38×10^{-5} were considered as suggestively linked, significantly linked genome-wide and highly significantly linked genome-wide, respectively. The 95% confidence intervals for the locations of the QTL were obtained by chi-square drop approximation (Mangin *et al.* 1994).

Results

Summary statistics for all traits and skewness coefficients for the variables analysed are shown in Table 1. Non-transformed values for FEC had a large variation, ranging from 150 to 65200 with a mean ± standard deviation of 12132.57 ± 8934.61. The ranges in variation for AVPCV (7.0–37.5% with mean ± standard deviation of 24.18 ± 4.59) and PCVST (22.0–50.5% with mean ± standard deviation of 34.28 ± 4.19) were smaller compared to PCVD (−3.0–31.0% with mean ± standard deviation of 10.10 ± 5.34). LWT ranged from 6.0 to 33.5 kg, with a mean ± standard deviation value of 18.38 ± 4.38. The positive skewness and kurtosis present in the FEC data are typical of this measured phenotype. The transformation methodology applied to these data reduced coefficients of asymmetry for all the variables. Expected values for skewness and kurtosis are zero for a normal phenotype distribution, and the Box–Cox and arcsine transformations generally decreased the absolute values of these parameters, thus improving data quality for subsequent QTL analysis.

High-quality genotypes for QTL detection in all six families were generated from a total of 172 markers covering 25

Table 1 Summary statistics of phenotypes after correction for fixed effects.

Trait	No. of observations	Mean ± SD	Mean ± SD of transformed data	Skewness of transformed data
AVFEC[1]	1063	12132.57 ± 8934.61	3.97 ± 0.32	−0.72
AVPCV[2]	1063	24.18 ± 4.59	0.24 ± 0.01	−0.61
PCVST[3]	1063	34.28 ± 4.19	0.26 ± 0.01	−0.20
PCVD[4]	1063	10.10 ± 5.34	0.21 ± 0.01	0.45
AVLWT[5]	1063	18.38 ± 4.38	–	–

[1]Average final faecal egg count (FEC) from measurements on two consecutive days at the end of pasture challenge.
[2]Average final of packed cell volume – PCV (%).
[3]PCV at the start of the pasture challenge (%).
[4]PCV decline on pasture (PCVST–AVPCV).
[5]Average final LWT (kg), non-transformed.
LWT, Live weight; PCVD, PCV from challenge start to end.

autosomes with the total map distance of 1560.7 cM, an average of six markers per chromosome, and an average marker interval resolution of 13.57 cM (Fig. S1). The two biggest gaps in coverage were because of a lack of informative markers on chromosome (Chr) 24 and a 56 cM gap on Chr 7. Marker interval resolution could have been finer; however, many markers failed screening because of poor amplification or the presence of null alleles segregating in the population. There was no evidence of segregation distortion in the backcross population, and GIC was mostly above 72%; however, some variation in this parameter was observed and validated along the chromosomes. The average value of PIC was 0.68, with markers *HAUT1* and *BM155* having the highest PIC value (0.90) and marker *IL12* the lowest (0.30).

Quantitative trait loci analysis using QXPAK revealed 18 significant effects (10% genome-wide significance) for the five traits analysed (Table 2). The majority of QTL (13) were associated with AVFEC, and all the other traits were associated with one QTL, except PCVST, which had two QTL. All QTL were similar in directional breed effects, as the favourable allele was always contributed by the Red Maasai. Among these QTL, AVFEC (Chr 3 and 6) and AVPCV (Chr 22) had effects reaching the 5% genome-wide significance

Table 2 QTL detection results for parasite indicator traits.

Trait	Chr	Position (95% CI)[1]	Markers flanking (position)	LR[2]	*P*-value[3]	a ± SE
AVFEC	1	243.0 (209.0–316.0)	*BM8246* (233.8)–*BM864* (256.1)	9.22	$2.39 \times 10^{-3\dagger}$	0.111 ± 0.038
	3	260.0 (237.0–277.0)	*CSAP39E* (259.7)–*BM2830* (279.5)	14.38	$1.79 \times 10^{-4*}$	0.168 ± 0.046
	6	45.0 (37.0–75.0)	*BM1329* (45.0)–*BMS360* (80.8)	15.91	$6.64 \times 10^{-5*}$	0.133 ± 0.035
	8	128.0 (104.0–128.0)	*MNS61A* (87.4)–*BMS1967* (128.0)	10.68	$1.08 \times 10^{-3\dagger}$	0.138 ± 0.044
	9	17.0 (1.0–34.0)	*ETH225* (9.4)–*CSSM66* (24.1)	11.70	$6.25 \times 10^{-4\dagger}$	0.120 ± 0.036
	13	98.0 (1.0–127.0)	*CTSBJ12* (98.2)–*BMS2319* (126.6)	8.71	$3.16 \times 10^{-3\dagger}$	0.059 ± 0.021
	14	54.0 (1.0–94.0)	*BMS2213* (33.9)–*HAUT14* (57.9)	18.94	$1.35 \times 10^{-5**}$	0.146 ± 0.034
	15	40.0 (1.0–70.0)	*BMS1004* (26.7)–*JAB8* (45.9)	13.28	$2.68 \times 10^{-4\dagger}$	0.139 ± 0.039
	16	13.0 (1.0–33.0)	Telomere–*BM1225* (13.2)	12.15	$4.91 \times 10^{-4\dagger}$	0.115 ± 0.034
	17	36.0 (1.0–127.0)	*URB048* (6.7)–*MAF209* (49.4)	8.78	$3.04 \times 10^{-3\dagger}$	0.108 ± 0.039
	22	40.0 (20.0–52.0)	*BM1314* (34.5)–*BM4505* (43.5)	28.11	$1.15 \times 10^{-7**}$	0.182 ± 0.035
	23	27.0 (7.0–41.0)	*BMS2270* (24.4)–*CSSM31* (31.2)	13.54	$2.33 \times 10^{-4\dagger}$	0.108 ± 0.030
	26	71.1 (61.0–72.0)	*POLBF17* (61.5)– *BM203* (71.1)	11.98	$5.37 \times 10^{-4\dagger}$	0.118 ± 0.035
AVPCV	22	40.0 (16.0–58.0)	*BM1314* (34.5)–*BM4505* (43.5)	10.47	$1.21 \times 10^{-3*}$	−1.874 ± 0.493
PCVST	3	254.0 (223.0–280.0)	*LS11* (238.4)–*CSAP39E* (259.7)	12.21	$4.76 \times 10^{-4\dagger}$	−2.370 ± 0.670
	18	1.0 (1.0–45.0)	Telomere–*BM3413* (22.3)	12.26	$4.62 \times 10^{-4\dagger}$	−1.251 ± 0.356
PCVD	22	41.0 (14.0–76.0)	*BM1314* (34.5)–*BM4505* (43.5)	11.45	$7.15 \times 10^{-4\dagger}$	1.729 ± 0.541
LWT	23	37.0 (16.0–50.0)	*AGLA* (35.6)–*ADCY1AP* (45.6)	9.51	$2.05 \times 10^{-3\dagger}$	−1.087 ± 0.348

[1]Positions are shown in cM, with approximate 95% confidence interval between brackets.
[2]LR: likelihood ratio test values.
[3]Nominal *P* values when testing QTL effect; †Suggestive linkage, *significant linkage genome-wide, **highly significant linkage genome wide.
a, additive QTL effect (Dorper – Red Maasai effect); SE: standard error; LWT, Live weight; PCVD, PCV from challenge start to end; QTL, quantitative trait loci.

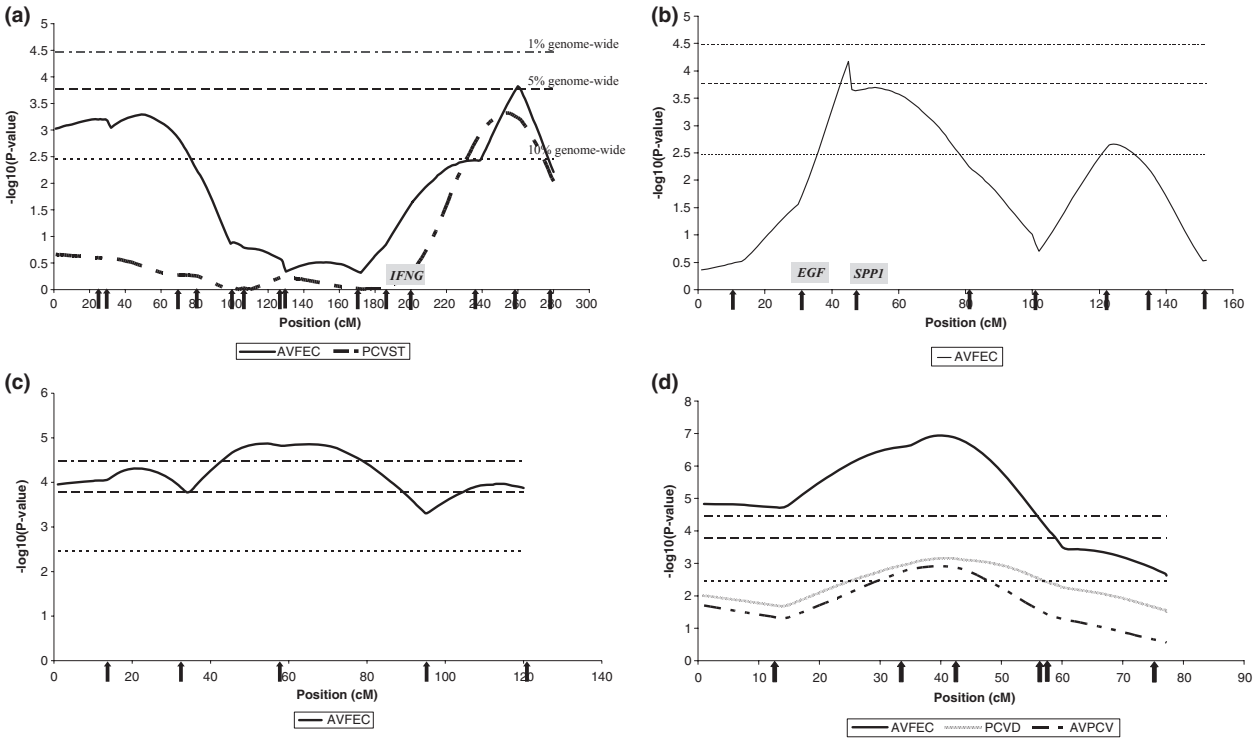

Figure 1 Profile plots of most significant parasite indicator QTL detected in the backcross sheep population. For all QTL profile plots the black vertical arrows on x-axis denote microsatellite marker positions along a chromosome and the y-axis represents the level of statistical significance: 2A) plot of Chr 3 QTL for AVFEC and PCVST and the relative position of interferon gamma (IFNG) gene is shown for reference, 2B) plot of Chr 6 QTL for AVFEC and relative map positions of epidermal growth factor (EPG) and secreted phosphoprotein 1 (SPP1) are shown, 2C) plot of Chr 14 QTL for AVFEC, and 2D) plot of Chr 22 QTL for AVFEC, PCVD, and AVPCV.

threshold, while only AVFEC (Chr 14 and 22) reached effects at the 1% genome-wide significance threshold. The QTL profile plot for Chr 3 actually suggests two QTL for AVFEC (Fig. 1a) that cover large intervals on the ends of the chromosome, while signal distal to the *interferon, gamma* (*IFNG*) locus (50 cM) overlaps with the QTL for PCVST. Likewise, the QTL plot for AVFEC on Chr 6 has a bimodal signal with the peak residing between the *epidermal growth factor* (*EGF*) and *secreted phosphoprotein 1* (*SPP1*) loci (Fig. 1b). This QTL accounts for 8% of the total additive genetic variance. The highest signals for AVFEC QTL on Chr 14 (Fig. 1c) and Chr 22 (Fig. 1d) were both centrally located on their respective chromosomes. The QTL on Chr 14 (54 cM), which is not well resolved, accounted for 16% of the total additive genetic variance, while the one on Chr 22 (40 cM) accounted for 10%. This AVFEC QTL on Chr 22 also had the largest likelihood ratio of all QTL detected in the study, and seemed to overlap QTL for AVPCV and PCVD, as shown by the matching contour plots (Fig. 1d), suggesting that the same QTL locus affected these 3 traits. On Chr 23, the QTL associated with AVLWT was also found to overlap with an AVFEC QTL (data not shown). Surprisingly, the AVLWT QTL had a positive additive effect derived from the Red Maasai allele and was significant at the 10% genome-wide threshold.

Discussion

Quantitative trait loci for indicators of endoparasite resistance were detected in a novel double backcross population. Although there are numerous other reports for parasite indicator QTL detection from sheep, our study contains several novel approaches and findings that should be useful in subsequent investigations aiming to resolve the effects of host genetic variation on endoparasite tolerance.

First, all other studies to date have been done without complete normalization of the phenotypic data for FEC. Normalization is a critical parameter for QTL detection, as the assumption of normality is central to typical approaches, but there is not much information about non-normality of data and its effect on the (co)variance components, genetic parameters and QTL mapping. Small values of kurtosis and asymmetry in the analysis of variance are important indicators of how the normality deviation can influence the estimates (Scheffe 1959). Skewness coefficients for the variables analysed are presented in Table 1. The estimate of parameter of transformation λ, obtained by maximum likelihood (ML), was different from zero, which indicated that the logarithmic transformation typically used for FEC data in QTL mapping was not optimal. Most QTL mapping methods assume that phenotypes are normally

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distributed, and this assumption is clearly violated for many measures of disease resistance (Tilquin *et al.* 2001). Therefore, improving normality should improve behaviour of the statistical tests. Aspects related to statistical analyses and data interpretation were discussed by Dominik (2005) and Tilquin *et al.* (2001). Davies *et al.* (2006) found the variance components estimates for FEC were very sensitive to the log transformation, but the transformation did not affect the QTL results. However, Silva *et al.* (2008) compared FEC data from cattle transformed by log and an extension of the Box–Cox methodology. Box–Cox transformation reduced coefficients of asymmetry, thereby improving the quality of FEC QTL mapping. In the present study, the QTL for Box–Cox transformed AVFEC detected on Chr 6 at the 5% genome-wise significant *P*-value (Fig. 1b) was not detected using raw and log transformation AVFEC data.

With respect to the QTL results, the four most highly significant QTL were identified for AVFEC on Chr 3, 6, 14 and 22. There are several studies showing evidence of QTL on Chr 3 near *IFNG* (Beh *et al.* 2002; Davies *et al.* 2006). This gene also appears to be a functional candidate for disease resistance, because neutralization of *IFNG* during infection causes a reduction in both crypt length and epithelial cell proliferation, suggesting a key role for *IFNG* nematode-associated alteration in epithelial architecture (Cliffe *et al.* 2007). Results reported previously for naturally infected Soay sheep (Coltman *et al.* 2001) further support the role of a gene conferring increased resistance to gastrointestinal nematodes being located at or near the *IFNG* gene. However, it is interesting to note that despite the fact that many studies have reported significant association between markers near the 5′ region of the gene and resistance, the phase of the resistance haplotype was not consistent (Raadsma *et al.* 2008). More recently, Beraldi *et al.* (2007) reported that the *IFNG* region did not produce any particular evidence of linkage in their study. This result is in agreement with our findings, where the maximum LR tests for AVFEC and PCVST (at position 260.0) on Chr 3 were located more than 60 cM from *IFNG*. There was little evidence of linkage at the *IFNG* locus (LRT = 1.6). Our results support the report by Moreno *et al.* (2006) of a QTL near position 275.0 and suggest that our Chr 3 QTL probably represents linkage to a gene distinct from *IFGN*. Failure to detect linkage near *IFNG* could be because of a lack of QTL segregating in this region, insufficient power of the current sample size, or lack of informative markers in linkage with the target region.

On Chr 6, the peak signal of the AVFEC QTL is located at 45 cM. There is no previous evidence for QTL associated with parasitic infection in this region between *SPP1* and *EGF* on Chr 6. The best comparison is with studies by Beraldi *et al.* (2007), who detected a suggestive QTL for logFEC at 74.0 cM, and Moreno *et al.* (2006), who reported two suggestive QTL associated with FEC at positions 18.0 and 73.0 cM. Interestingly, the weaker portion of the bimodal signal (Fig. 1b) in our study corresponds with a QTL identified by Beh *et al.* (2002) on Chr 6 (LOD score = 4.2) at 130 cM for resistance to *T. colubriformis* in Merino sheep families, and findings by Marshall *et al.* (2009), who reported a within-family QTL for logFEC located at 151.2 cM.

The location of our QTL on Chr 6 contains many functional gene candidates known to influence immune response. One of these is *SPP1* (previously known as *osteopontin, OPN*) and is an active participant in physiological and pathological processes that include wound healing, burn turnover, tumorigenesis, inflammation, ischaemia and immune responses (Wang & Denhardt 2008). *SPP1* is expressed by many different cell types, including macrophages, neutrophils, dendritic cells, NK cells, and T and B lymphocytes. Increased expression in response to injury and inflammation is associated with increased cell mobilization, survival and activity, and with elevated concentrations of SPP1 protein in tissue fluids and plasma. Detection of *SPP1* expression has potential diagnostic and prognostic value for monitoring inflammatory disease progression in the intestines (Sodek *et al.* 2006). A second candidate gene found about 20 cM from our QTL is *EGF*, a key mediator of cell communication during animal development and homeostasis. *EGF* has been implicated in the host–parasite molecular dialogue and parasite development (Dissous *et al.* 2006). In humans, *EGF* directly induces epithelial cell proliferation, and lack of amphiregulin delayed expulsion of the nematode *Trichuris muris* (Zaiss *et al.* 2006).

On Chr 14, Davies *et al.* (2006) found significant associations between three different phenotypic indicators of nematode resistance at positions 100, 103 and 104 cM. This study suggested that the QTL effect appeared to be the same across the three traits. However, our findings of an AVFEC QTL at 54.0 cM (LRT = 18.94) more closely correspond to QTL for FECs detected by Moreno *et al.* (2006).

The most significant QTL for AVFEC found in this study (LRT = 28.11) is located at position 41.0 cM on Chr 22. Furthermore, significant associations for AVPCV and PCVD were found at positions 40.0 cM and 41.0 cM, respectively, suggesting that these QTL are affected by the same underlying causative genetic variation. A similar result for AVFEC was found by Marshall *et al.* (2009) on Chr 22, but no other studies have detected any parasite indicator QTL at this position.

The evidence for QTL detected on Chr 1, 5, 6, 8, 9, 13, 15, 16, 17, 18, 20, 23 and 26 was less significant, and QTL were detected for only one trait. Therefore, the QTL plots were not shown. QTL mapping in an F_2 population produced from Gulf Coast Native (resistant) and Suffolk (susceptible) crosses has found suggestive QTL regions for AVFEC and PCV on Chr 1, 3 and 19 (Cockett *et al.* 2005). Further fine-mapping then identified QTL on Chr 9 and 19

(Hadfield *et al.* 2008, 2009). These findings do not correlate well with any of our results.

According to Sayers & Sweeney (2005), genes of known function in or surrounding significant regions on Chr 1 and 6 have not yet been identified, thus preventing any elaboration on their potential phenotypic role in resistance. With the exception of OAR20, where the major histocompatibility complex (MHC) is found in two regions, genes with a potential role in resistance have not been previously detected on Chr 5, 8, 9, 13, 15, 16, 17, 18, 23 and 26.

In conclusion, our results support the hypothesis that some QTL on Chr 3, 6, 14 and 22 influence variation in parasitic infection and immune response. We found evidence for a new QTL on Chr 6, between *EGF* and *SPP1*, which has not been reported previously. The most significant QTL for AVFEC, AVPCV and PCVD were located close to the same position on Chr 22, suggesting that they may actually be a single QTL associated with all three traits.

Eventual fine-mapping of the QTL or identification of the gene involved in resistance to GI parasites may have an important economic impact on sheep breeding. However, it is clear from this study and the other QTL studies discussed in this paper that the genetic control of resistance to internal parasites in sheep is a polygenic trait. Thus, we agree with Marshall *et al.* (2009), who stated that 'a panel of QTL will be required for significant genetic gains to be achieved within industry via marker-assisted selection'.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Reference map locations of 172 microsatellite markers used for QTL analysis.

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