## Accepted Manuscript

Title: Transcriptional profiling of the ovine abomasal lymph node reveals a role for timing of the immune response in gastrointestinal nematode resistance



Author: Kathryn M. McRae Barbara Good James P. Hanrahan Matthew S. McCabe Paul Cormican Torres Sweeney Mary J. O'Connell Orla M. Keane

PII:	\$0304-4017(16)30179-0
DOI:	http://dx.doi.org/doi:10.1016/j.vetpar.2016.05.014
Reference:	VETPAR 8014
To appear in:	Veterinary Parasitology
Received date:	10-12-2015
Revised date:	3-5-2016
Accepted date:	12-5-2016

Please cite this article as: McRae, Kathryn M., Good, Barbara, Hanrahan, James P., McCabe, Matthew S., Cormican, Paul, Sweeney, Torres, O'Connell, Mary J., Keane, Orla M., Transcriptional profiling of the ovine abomasal lymph node reveals a role for timing of the immune response in gastrointestinal nematode resistance.Veterinary Parasitology http://dx.doi.org/10.1016/j.vetpar.2016.05.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 2 3 4	Transcriptional profiling of the ovine abomasal lymph node reveals a role for timing of the immune response in gastrointestinal nematode resistance
5	Kathryn M. McRae <sup>1,2†</sup> , Barbara Good <sup>3</sup> , James P. Hanrahan <sup>3</sup> , Matthew S. McCabe <sup>1</sup> ,
6	Paul Cormican <sup>1</sup> , Torres Sweeney <sup>4</sup> , Mary J. O'Connell <sup>2</sup> & Orla M. Keane <sup>1§</sup>
7	
8	<sup>1</sup> Animal & Bioscience Department, Teagasc, Grange, Dunsany, Co. Meath, Ireland.
9	<sup>2</sup> Bioinformatics & Molecular Evolution Group, School of Biotechnology, Dublin City
10	University, Glasnevin, Dublin 9, Ireland.
11	<sup>3</sup> Animal & Bioscience Department, Teagasc, Athenry, Co. Galway, Ireland.
12	<sup>4</sup> School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4,
13	Republic of Ireland
14	Current address: <sup>†</sup> AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand
15	<sup>§</sup> Corresponding author
16	
17	Email addresses:
18	KMM: <u>kathryn.mcrae@agresearch.co.nz</u>
19	BG: <u>barbara.good@teagasc.ie</u>
20	JPH: <u>sjohanrahan@gmail.com</u>
21	MMC: matthew.mccabe@teagasc.ie
22	PC: paul.cormican@teagasc.ie
23	TS: <u>torres.sweeney@ucd.ie</u>
24	MJO'C: <u>mary.oconnell@dcu.ie</u>
25	OMK: orla.keane@teagasc.ie

- Nematode resistant and susceptible lambs were identified using a previously developed model
- Resistant lambs had more immature and shorter worms
- Genes involved in the inflammatory response, attraction of T lymphocytes and binding of leukocytes were more highly expressed in resistant animals at 7 dpi and in susceptible animals at 14 dpi indicating that resistant lambs appear to generate an earlier immune response
- No SNP in differentially expressed genes were significantly associated with nematode resistance but SNP in 2 genes (*SLC30A2* and *ALB*) were suggestively associated

## 26 Abstract

27 Gastrointestinal nematodes are a serious cause of morbidity and mortality in grazing 28 ruminants. The major ovine defence mechanism is acquired immunity, with protective 29 immunity developing over time in response to infection. Nematode resistance varies 30 both within and between breeds and is moderately heritable. A detailed understanding 31 of the genes and mechanisms involved in protective immunity, and the factors that 32 regulate this response, is required to aid both future breeding strategies and the 33 development of effective and sustainable nematode control methods. The aim of this 34 study was to compare the abomasal lymph node transcriptome of resistant and 35 susceptible lambs in order to determine biological processes differentially expressed 36 between resistant and susceptible individuals.

37 Scottish Blackface lambs, with divergent phenotypes for resistance, were 38 challenged with 30,000 Teladorsagia circumcincta larvae (L3), and abomasal lymph 39 nodes recovered at 7 and 14 days post-infection (dpi). High-throughput sequencing of 40 cDNA from the abomasal lymph node was used to quantitatively sample the 41 transcriptome with an average of 32 million reads per sample. A total of 194 and 144 42 genes were differentially expressed between resistant and susceptible lambs at 7 and 43 14 dpi respectively. Differentially expressed networks and biological processes were 44 identified using Ingenuity Pathway Analysis. Genes involved in the inflammatory 45 response, attraction of T lymphocytes and binding of leukocytes were more highly 46 expressed in resistant animals at 7 dpi and in susceptible animals at 14 dpi indicating 47 that resistant animals respond to infection earlier than susceptible animals. Twenty-48 four Single Nucleotide Polymorphisms (SNP) within 11 differentially expressed 49 genes, were tested for association with gastrointestinal nematode resistance in the

50 Scottish Blackface lambs. Four SNP, in 2 genes (*SLC30A2* and *ALB*), were 51 suggestively associated with faecal egg count.

In conclusion, a large number of genes were differentially expressed in the abomasal lymph node of resistant and susceptible lambs responding to gastrointestinal nematode challenge. Resistant Scottish Blackface lambs appear to generate an earlier immune response to *T. circumcincta*. In susceptible lambs this response appears to be delayed. SNP in 2 differentially expressed genes were suggestively associated with faecal egg count indicating that differentially expressed genes may be considered candidate loci for mediating nematode resistance.

## 59 Keywords

60 Scottish Blackface; Sheep; *Teladorsagia circumcincta*; Host-parasite interaction;
61 Transcriptome.

## 63 **1. Introduction**

64 Gastrointestinal nematodes (GIN) are a serious cause of morbidity and mortality in 65 grazing ruminants. Infected lambs have a reduced ability to absorb nutrients from the 66 gastrointestinal tract, resulting in ill-thrift and, occasionally, death. Sub-clinical infection adds to the production losses in the form of reduced growth rate and light, 67 68 under-finished carcasses. Anthelmintic drenching has been the method of choice for nematode control for the last 50 years; however, consumer concerns about food 69 70 products from animals subjected to chemical treatment, combined with the inevitable 71 evolution of anthelmintic resistant nematodes, means alternative, sustainable methods 72 of parasitic nematode control are required.

Resistance to GIN is moderately heritable  $(h^2 \sim 0.3)$  (Bishop and Morris. 73 74 2007; Safari et al., 2005), therefore a sustainable method of nematode control is to 75 select for genetically resistant individuals (Kemper et al., 2009). Selection using 76 phenotypic traits, such as faecal egg count (FEC), requires prior exposure to GIN, 77 whereas selection could be simplified through the identification of molecular markers. 78 A detailed understanding of the genes and mechanisms involved in expressing a 79 resistant phenotype and the factors that regulate this response would facilitate the 80 identification of candidate markers.

81 Transcriptome analysis is a powerful method for the identification and 82 quantification of genes expressed during a physiological perturbation. A number of 83 previous studies have been undertaken to characterise the duodenal (Diez-Tascon et 84 al., 2005; Keane et al., 2007; Keane et al., 2006), abomasal mucosal (Knight et al., 85 2011; Rowe et al., 2009) and lymph node and lymph fluid transcriptome (Andronicos 86 et al., 2010; Gossner et al., 2013; Knight et al., 2010; MacKinnon et al., 2009) and

87 have led to the identification of genes and biological processes associated with the 88 host response to GIN. As a result of these studies, a number of pathways have been 89 postulated to be involved in the development of a resistant phenotype; however, no 90 clear consensus has emerged. In Perendale selection lines, susceptible lambs were 91 found to have increased intestinal mucosal expression of genes involved in the stress 92 response, while resistant animals had increased expression of Major Histocompatibility Complex (MHC) class II, free radical scavenging and fatty acid 93 94 metabolism genes (Keane et al., 2007; Keane et al., 2006). Transcriptomic analysis of 95 the abomasal lymph node of Texel (resistant) and Suffolk (susceptible) lambs 96 suggested that a balanced T helper (Th) cell response was associated with resistance 97 (Ahmed, 2013). A comparison of the abomasal lymph node transcriptome of resistant 98 and susceptible Scottish Blackface lambs also identified Th cell differentiation and 99 polarisation as important in the development of a resistant phenotype (Gossner et al., 100 2013). Differences between the studies may reflect biological or technical variation in 101 the experimental design such as tissue sampled, lamb age, nematode exposure history, 102 the magnitude and species of the nematode challenge, or the transcriptomic platform. 103 Alternatively, the differences may reflect physiological differences between breeds 104 and individuals in how they develop resistance.

105 Resistance to GIN may be manifested by controlling worm burden, worm 106 fecundity or a combination of both (Stear et al., 1996b). The majority of previous 107 studies concerning gene expression in resistant and susceptible animals have been 108 based on a model where resistant and susceptible animals differ significantly in worm 109 burden (Ahmed, 2013; Gossner et al., 2013; Keane et al., 2007; Keane et al., 2006; 110 Pernthaner et al., 2005; Zaros et al., 2014). However, the genes and pathways 111 involved in regulating worm fecundity may differ from those involved in controlling

112 worm burden. We previously described a method to reliably identify repeatable within-breed variation in the ability of Scottish Blackface lambs to resist GIN 113 114 infection (McRae et al., 2014). Resistant lambs were found to display lower FEC, 115 lower worm fecundity and a higher level of anti-nematode IgA in both serum and 116 mucosa. The physiological response to infection, as indicated by anti-nematode 117 antibody levels, haematology and pepsinogen, was most pronounced at 7 and 14 days post-infection (dpi), although the phenotype (reduced FEC) was not yet evident at this 118 119 time point.

120 The aim of the present study was to use high-throughput sequencing of cDNA 121 to sample the transcriptome of the abomasal lymph node of Scottish Blackface lambs 122 with divergent phenotypes for GIN resistance in order to identify genes and biological 123 processes associated with the ability to express resistance. In this breed, repeatable differences among individuals in FEC, were positively associated with both increased 124 125 worm burden and increased worm fecundity (McRae et al., 2014; Stear et al., 1995). 126 Differentially expressed (DE) genes were considered candidate genes for mediating 127 resistance and markers in these genes were tested for association with FEC in a larger 128 Scottish Blackface cohort.

## 130 **2. Materials and Methods**

### 131 2.1 Ethical approval

The animal procedures described in this study were conducted under experimental
licence from the Irish Department of Health in accordance with the Cruelty to
Animals Act 1876 and the European Communities (Amendments of the Cruelty to
Animals Act 1976) Regulations, 1994.

136 2.2 Animals

Purebred male Scottish Blackface lambs (n = 92) were sourced from the flock at the
Teagasc Hill Sheep Farm, Leenane, Co. Mayo in 2010. Lambs were managed from
birth on improved lowland pasture where the major nematode species is *Teladorsagia circumcincta* (B. Good, unpublished data). All lambs received an oral benzimidazole
anthelmintic treatment at 5 weeks of age to control *Nematodirus battus* infection.

Flock FEC (eggs per gram (epg)) was monitored weekly, from when lambs 142 143 were approximately 8 weeks of age, using the FECPAK method (Fecpak). Eggs were 144 distinguished as *Nematodirus* spp. (FEC<sub>NEM</sub>) and 'other trichostrongyles' spp (FEC $_{OT}$ ). When FEC $_{OT}$  reached approximately 600 epg the lambs were individually 145 146 sampled twice (FEC1A and FEC1B), 1 week apart, and FEC was determined for each 147 sample using the modified McMaster method (Anon, 1986).  $FEC1A_{OT}$  and  $FEC1B_{OT}$ 148 were averaged to give FEC1<sub>OT</sub>, the first phenotypic measurement of resistance. 149 Following FEC1B the lambs were treated with a non-persistent macrocyclic lactone 150 (ML, Oramec, Merial Animal Health Ltd) in accordance with manufacturer's 151 recommendations. Flock FEC was again monitored weekly until FEC<sub>OT</sub> reached 152 approximately 600 epg when 2 more FEC (1 week apart) per individual were

153 completed (FEC2A and FEC2B) and the average computed to generate  $FEC2_{OT}$ , the 154 second phenotypic measurement of resistance. This cohort of animals constituted 155 grazing group 1. This process was replicated in 2011 with male (n = 76) and female 156 (n = 90) lambs in grazing groups 2 and 3, resulting in 2 phenotypic FEC 157 measurements from 258 animals which were used for genetic association studies.

### 158 2.3 Experimental infection

159 For the animals born in 2010 (n = 92), individual animal values for  $ln(FEC_{OT} + 25)$ 160 were used to identify the most resistant (subsequently known as "LowFEC") and 161 susceptible (subsequently known as "HighFEC") lambs, using mixed model procedures (SAS® v9.1). Data for each natural infection (FEC1<sub>OT</sub> and FEC2<sub>OT</sub>) were 162 163 analysed separately using a model that included rearing type (single or twin) and 164 sample date (A or B sample of round) as fixed effects and animal as a random term. To get the selection differential for each animal, the estimated animal effect from each 165 166 round was scaled by the standard error of prediction and averaged across rounds. 167 These differentials were used to select 10 HighFEC and 10 LowFEC animals. Five sires were used in the flock; all 5 had progeny selected as HighFEC while 3 sires had 168 169 progeny selected as LowFEC.

The selected lambs (n = 20) were cleared of helminth infection with a nonpersistent ML (Oramec, Merial Animal Health Ltd), in accordance with manufacturer's recommendations, and housed on straw bedding until slaughter, with free access to water and 600 g commercial lamb ration per head per day. All lambs were free of helminth infection for a minimum of 5 weeks prior to the experimental infection (based on FEC measurements on 3 consecutive days). All lambs received an oral challenge of approximately 30,000 *T. circumcincta* larvae (L3) at 31 (range 29 –

32) weeks of age. Lambs (5 per phenotype) were slaughtered at 7 and 14 dpi by
electrical stunning followed immediately by exsanguination. The experimental design
is summarised in Figure 1.

180 2.4 Phenotypic measurements and analysis

181 Worm burden, female worm length, haematology variables and anti-nematode 182 antibody level in both serum and abomasal mucosa were determined from samples 183 taken at slaughter, as previously described (McRae et al., 2014). As all worms 184 recovered at 7 dpi were immature and could not be sexed, worm length was measured 185 at 14 dpi only. For one animal (LowFEC) all worms recovered at 14 dpi were immature, for the remaining animals the mean number of female worms measured 186 187 was 77 (range 51 - 135). Log transformations were performed on worm burden data 188  $(\ln(X + 25))$  to stabilise the variance. Data were analysed using the Proc MIXED of 189 SAS® (v9.1) to fit a model that had effects for phenotype (HighFEC or LowFEC), dpi 190 and their interaction.

### 191 2.5 Tissue collection and RNA extraction

192 Abomasal lymph node tissue, recovered at slaughter, was immediately cut into pieces, approximately 0.5 cm<sup>3</sup>, and submerged in 10 volumes of RNAlater® (Ambion). This 193 194 was stored overnight at 4 °C followed by long-term storage at -80 °C. Total RNA was 195 extracted from the tissue using Sigma TRI Reagent® (Sigma Aldrich, UK) according 196 to the manufacturer's instructions. Small RNAs (<200 nucleotides) and residual 197 genomic DNA were removed with the RNeasy Mini Kit (Qiagen, Germany) and an in-solution DNase digestion (RNase-free DNase set; Qiagen, Germany) according to 198 199 the manufacturer's instructions. RNA quality was assessed using an Agilent® RNA 200 6000 Nano Assay on a 2100 Bioanalyzer, and total RNA was quantified using the

201 NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, UK). All 202 samples had a RIN value  $\geq 8.5$  and a 28S:18S ratio of  $\geq 1.5$ .

### 203 2.6 Library preparation and sequencing

Illumina TruSeq<sup>™</sup> libraries were prepared following the TruSeq<sup>™</sup> RNA sample 204 205 preparation v2 guide for total RNA (Part #15026495 Rev. B) with the following 206 modifications: (i) the number of PCR cycles was reduced to 10 to minimise 207 overcycling and (ii) the PCR products were purified using a Qiagen MinElute column 208 rather than AMPure XP beads to avoid bead contamination. Libraries were visualised 209 using an Agilent® DNA 1000 assay on a 2100 Bioanalyzer, and quantified using the 210 Qubit® dsDNA BR assay (Invitrogen, UK) according to the manufacturer's 211 instructions. The indexed cDNA libraries containing the specific Illumina TruSeq 212 adapters were sent to GATC Biotech (Kontanz, Germany), where they were 213 sequenced on an Illumina HiSeq2000 with 50 bp paired-end reads.

### 214 2.7 Bioinformatics analysis

215 Trim Galore (v0.3.3) (TrimGalore), which utilises Cutadapt (v1.2.1), was applied to 216 the RNAseq reads using the default settings for paired-end data. Reads with a median 217 Phred-scaled quality score below 20 were removed. Trimmed reads were mapped to 218 the ovine genome (OARv3.1) (Jiang et al., 2014) using STAR (v2.3) (Dobin et al., 219 2013), with the Ensembl Ovis aries transcriptome annotation (release 74) supplied. 220 Only uniquely mapped reads with a maximum of 2 mismatches to the reference 221 genome were retained for expression analysis. The mapped reads were used to 222 estimate raw counts per gene using HTSeq (v0.5.3p3) (HTSeq) with the union overlap 223 resolution mode. The between group analysis (BGA) function from the Bioconductor 224 package MADE4 (v1.42.0) (Culhane et al., 2005) was used to visualize the samples

225 based on transcriptomic profiles. The Bioconductor package EdgeR (v3.0.8) (Robinson et al., 2010) was run within R software (v3.0.2) to analyse differential 226 227 expression of read counts. Comparisons were made between HighFEC and LowFEC 228 animals at either 7 or 14 dpi, or within phenotype over time between 7 and 14 dpi. 229 Low expression tags were filtered, keeping only genes that achieved at least 1 count 230 per million in at least 5 samples. Trimmed mean of M-values normalisation 231 (Robinson and Oshlack, 2010) was used to account for differences in RNA 232 composition between samples. Data were analysed using both common and tagwise 233 dispersions. To account for multiple testing, genes were filtered using a Benjamini 234 and Hochberg false discovery rate (FDR) (Benjamini and Hochberg, 1995) of  $\leq 0.1$  or 235  $\leq 0.05$  for tagwise and common dispersion analyses, respectively. All genes identified 236 as DE using common dispersion estimates were included in pathway analysis. Pre-237 calculated 1-to-1 Human orthologs (Ensembl release 74) were obtained using 238 Ensembl's Biomart tool (Biomart). Ingenuity® Systems Pathway Analysis (IPA; 239 Ingenuity Systems, Redwood City, CA, USA; v18841524) was used to identify the 240 top networks, canonical pathways, diseases and functions from DE genes.

### 241 2.8 cDNA synthesis and RT-qPCR

First-strand cDNA synthesis from 1.5  $\mu$ g of total RNA was carried out using the High Capacity cDNA reverse transcription kit (Applied Biosystems) according to the manufacturer's instructions. Quantitative PCR was performed in triplicate in 20  $\mu$ l reactions with 10  $\mu$ l 2 X Fast Sybr Green Master Mix (Applied Biosystems), 1  $\mu$ l of forward and reverse primer (300 nM each) and 1  $\mu$ l of cDNA and reverse transcriptase in an Applied Biosystems Fast 7500 instrument. Reactions were denatured at 95 °C for 20 s then cycled 40 times at 95 °C for 3 s and 60 °C for 30 s.

249 A dissociation analysis was carried out at the end of the reaction to ensure a single 250 product was generated. Reaction efficiencies for each primer pair were calculated 251 using a 1:2 dilution series over 5 points and only those between 0.9 and 1.1 were 252 retained for analysis. Variation in PCR efficiency was corrected for and expression levels were normalised to that of the reference genes GAPDH, H3F3A and YWHAZ 253 254 using GenEx (v.3.6.170). RNASeq and qPCR data were compared by calculating the 255 correlation coefficient for each gene. Primer sequences and correlations can be found 256 in Supplementary file 1.

### 257 2.9 SNP genotyping and analysis

258 Whole blood was collected from 258 Scottish Blackface lambs for which FEC 259 phenotypes were recorded (Figure 1). Genomic DNA was extracted from 9 mL of blood using the high salt method (Montgomery and Sise, 1990). In some cases blood 260 261 was frozen, which results in lysis of the white blood cells; for these samples genomic 262 DNA was extracted from 400 µL of blood using the Maxwell® 16 Research System 263 (Promega, UK), according to the manufacturer's instructions. DNA could not be 264 extracted from 21 samples. Consequently, DNA was available for 237 Scottish 265 Blackface lambs.

SNP information derived from the Ovine Infinium® HD SNP BeadChip was extracted for 11 of the 13 genes DE between HighFEC and LowFEC animals using tagwise dispersion (Supplementary File 2). The remaining 2 genes were on scaffolds or contigs, so their genomic location was unknown. These were discarded from further analysis. SNP were further classified as in coding or non-coding regions. SNP were prioritised for genotyping using the following criteria: 1) at least one SNP in

each gene of interest, 2) SNP in a coding region of the gene of interest, and 3) SNP
polymorphic in the Scottish Blackface RNA-Seq data set.

Twenty-four SNP in 11 DE genes were genotyped in 237 Scottish Blackface animals by Sequenom GmbH (Germany) using the MassARRAY® system. All genotyped SNP had a minor allele frequency >0.02 and a call rate >90%, and as a consequence were included in downstream analysis. No SNP deviated from Hardy Weinberg equilibrium.

SNP effects for FEC traits (FEC1 and FEC2 for *Nematodirus* and for 'other trichostrongyles') were estimated in ASReml (Gilmour et al., 2009) by fitting the SNP, one at a time, as fixed effects. The model also included sex and grazing group as fixed effects. After Bonferroni correction, the significance level thresholds were P < 2.08  $\times$  10<sup>-3</sup> and P < 4.17  $\times$  10<sup>-2</sup> for genome-wide significance (P < 0.05) and suggestive significance (that is, one false positive per genome scan), respectively.

## 285 **3. Results**

## 286 3.1 Phenotypic measurements

Scottish Blackface lambs at the extremes of the distribution of parasite resistance and susceptibility were identified. The selected lambs, chosen for high (n = 10) or low (n = 10) FEC had an average (range) FEC of 1,373 (1,000-1,661) and 216 (85-267) epg, respectively (Figure 2A). This selection method has previously been demonstrated to reliably identify resistant and susceptible individuals (McRae et al., 2014) and the difference in selection differential between High and LowFEC lambs was 3.18 standard deviation units.

294 After a controlled challenge with T. circumcincta there was no significant 295 difference between HighFEC and LowFEC animals in worm burden, at either 7 or 14 296 dpi. This was expected, as resistance in this flock primarily manifests as reduced 297 worm fecundity in resistant individuals, although worm burden may also be a contributing factor (McRae et al., 2014). In total, 75% of worms recovered from the 298 299 abomasum of HighFEC lambs had developed to the L5 stage by 14 dpi with the 300 remainder at L4. This compared to 54% L5 in the LowFEC lambs; however, the 301 difference in adults as a proportion of total worms was not significant (Mann-Whitney 302 U test, P = 0.15). Excluding the LowFEC lamb which carried only immature larvae, 303 the mean (s.e) length of female T. circumcincta in HighFEC and LowFEC animals 304 was 6.38 (0.25) and 5.59 (0.27) mm, respectively (P = 0.07). In agreement with our 305 previous study, the number of circulating basophils was higher in LowFEC animals in 306 comparison to HighFEC animals (Figure 2B; P = 0.03). Phenotypic differences for 307 other haematology measurements were not significant. The weight of the abomasal lymph nodes was also higher in LowFEC lambs than in HighFEC lambs (means of 308

- 309 4.86 g and 3.65 g respectively; P = 0.048) while the level of IgA specific for T.
- 310 *circumcincta* was significantly higher at 7 dpi in both serum (Figure 2C; P = 0.002)
- 311 and mucosa (Figure 2D; P = 0.002).

### 312 3.2 RNA Sequencing

313 High throughput sequencing of the 20 ovine abomasal lymph node RNA samples 314 resulted in 790,415,623 paired-end reads. Approximately 1% of reads were excluded 315 from downstream analysis due to low quality (Phred score < 20). An average of 316 32,573,191 reads per sample (83%) mapped to a unique region of the ovine genome, 317 in excess of the 20 to 25 million mappable reads recommended by the ENCODE 318 Consortium for gene expression analysis (ENCODE). Of the uniquely mapped reads, 319 an average of 18,040,721 (55%) aligned to a known feature and an average of 320 14,205,416 (44%) did not align to a known gene. The remaining 1% of reads could 321 have been assigned to more than one feature, and were thus classified as ambiguous. 322 A between-group analysis (BGA) plot, based on correspondence analysis of overall 323 gene expression values, is shown in Supplementary File 3.

#### 324 3.3 Differential gene expression

The number of DE genes detected is shown in Table 1. Genes detected as DE using tagwise dispersion are reported in Table 2 while the complete list of genes identified as DE using common dispersion are in Supplementary File 4. Tagwise dispersion ranks genes more highly when counts are consistent between individuals within a group, while common dispersion is more likely to rank genes as DE even when they are highly variable within a group.

331 3.4 Genes DE between HighFEC and LowFEC animals

332 A total of 13 genes were identified as DE between HighFEC and LowFEC animals 333 using tagwise dispersion - 7 DE at 7 dpi and 6 DE at 14 dpi (Table 1); 5 (38%) were 334 novel protein coding genes and 3 (23%) had been identified in previous studies (Table 335 2). These percentages are in line with those identified using the common dispersion analysis where 126 DE genes (37%) were novel protein-coding genes and 91 DE 336 337 genes (27%) had been identified in previous studies. Human 1-to-1 orthologs were 338 found for 60% of the common dispersion DE genes (Supplementary File 4) and these 339 orthologs were used as input for IPA analysis.

340 The top network for genes DE between HighFEC and LowFEC animals at 7 341 dpi was 'Cell-To-Cell Signalling and Interaction, Cellular Movement, Immune Cell 342 Trafficking' while the top network at 14 dpi related to 'Cellular Assembly and 343 Organisation, Lipid Metabolism and Small Molecule Biochemistry' (Table 3). Two 344 canonical pathways were also significant in the HighFEC vs LowFEC comparison at 345 7 dpi (Table 4). These pathways included a number of chemokine (C-X-C motif) 346 ligands (CCL and CXCL) and receptors (CCR) that were more highly expressed in 347 LowFEC animals at 7 dpi.

348 3.5 Immune response over time

349 Changes in the immune response to GIN over time post-infection were examined 350 within phenotype (HighFEC or LowFEC) by looking at the transcriptional profiles of 351 animals slaughtered at 7 dpi compared to those slaughtered at 14 dpi. A total of 21 DE 352 genes were detected using tagwise dispersion, 7 were DE in HighFEC animals and 14 353 in LowFEC animals. Of these genes, 13 (61%) were novel protein coding genes while 5 (24%) had been identified in previous studies (Table 2). Of the 387 DE genes found 354 355 using common dispersion estimates, 138 (36%) were novel protein-coding genes and 356 110 (28%) had been identified in previous studies.

The networks identified by IPA using genes DE in HighFEC or LowFEC animals over time are given in Table 3. The network 'Cell-To-Cell Signalling and Interaction, Cellular Movement, Immune Cell Trafficking' was the second highest network for LowFEC animals. Six canonical pathways were significantly DE between 7 and 14 dpi in LowFEC animals (Table 4). This was once again primarily due to the increased expression of a number of chemokine ligands and receptors in the LowFEC animals at 7 dpi.

364 3.6 Visualisation across multiple analyses using Ingenuity Pathway Analysis

365 The Comparison Analysis in IPA was used to compare results from the HighFEC vs LowFEC analyses at both 7 and 14 dpi. The top diseases and biological functions 366 367 (Figure 3) and the top upstream regulators (Figure 4) were compared. At 7 dpi, functions including 'inflammatory response', 'attraction of T lymphocytes' and 368 369 'synthesis of reactive oxygen species' were increased in LowFEC animals (Figure 3). 370 In contrast, genes related to 'cancer' were more highly expressed in HighFEC 371 animals. By 14 dpi, genes relating to 'inflammatory response' and 'synthesis of 372 reactive oxygen species' were more highly expressed in HighFEC lambs. At 7 dpi, 373 expression of genes downstream from Tumour Necrosis Factor (TNF) and a number of members of the interferon (IFN) group of signalling proteins were increased in 374 375 LowFEC animals. Genes downstream of these cytokines were not increased in 376 HighFEC animals until 14 dpi.

377 3.7 Validation of expression

In order to validate the results of the RNA sequencing, a panel of 8 DE genes were chosen for validation by RT-qPCR. These were *ALB*, *ASZ1*, *CXCL11*, *GABBR2*, *GSDMA*, *LYVE1*, *MFI2* and *STPG1*. The gene expression pattern, in terms of direction and magnitude of 7 of the 8 genes was reproducible by qPCR

(Supplementary file 1). For one gene (*LYVE1*) the direction of change was the same
for the RNASeq and qPCR but the correlation was not significant.

### 384 3.8 SNP analysis

385 The results of an association analysis between SNP in DE genes and FEC are shown 386 in Table 5. The results yielded a range of significance for each individual SNP; 387 however, no single SNP reached significance after Bonferroni correction for multiple comparisons (Abdi, 2007). Four SNP were, however, suggestively associated with 388 389 FEC2<sub>OT</sub>: 2 coding SNP within SLC30A2, and 2 non-coding SNP within ALB. Both 390 SNP within SLC30A2 code for missense variants; however, SNP OAR2 239929582 391 is classified as 'tolerated' by the SIFT algorithm (score = 1) (Kumar et al., 2009), 392 whereas SNP OAR2\_239931409 is classified as 'deleterious' (score = 0). The latter 393 SNP has a minor allele (A) frequency of 0.03 within the set of individuals studied, 394 with only 14 out of 237 animals heterozygous and no animal homozygous for this 395 allele. However, the allele was not out of Hardy-Weinberg equilibrium (P = 0.90).

## 397 **4. Discussion**

### 398 4.1 Phenotypic differences between resistant and susceptible lambs

399 We have previously described a method to reliably identify repeatable within-breed 400 variation in the ability of Scottish Blackface lambs to resist GIN infection (McRae et 401 al., 2014). Resistant lambs had lower FEC, primarily due to lower worm fecundity. 402 This method was used to generate the HighFEC and LowFEC lambs for the present 403 study. As the host response to infection was most pronounced in the first 2 weeks of 404 infection (McRae et al., 2014), the lambs were euthanized before the infection became 405 patent meaning we could not measure FEC in the lambs; however, resistant lambs 406 tended to have more immature larvae and shorter worms in addition to a higher 407 number of circulating basophils. This is consistent with our previous study, and 408 induction of basophils is known to be a feature of the anti-helminth response (Allen 409 and Maizels, 2011). Recent studies indicate that basophils play a role in regulating 410 acquired immunity by initiating Th2 cell differentiation, as well as in amplifying the 411 humoral memory response (Karasuyama et al., 2011). The weight of the abomasal 412 lymph node was also higher in resistant lambs, which may reflect increased cellular 413 recruitment to the lymph node.

### 414 4.2 Response to GIN infection in phenotypes divergent for resistance

At 7 dpi *ALB* was more highly expressed in the HighFEC group. This gene has been associated with GIN infection in 4 separate studies. Serum albumin, the main protein of plasma, is a carrier protein for steroids, fatty acids and thyroid hormones, and functions as a regulator of the colloidal osmotic pressure of blood. ALB is a negative acute phase protein, which has been shown to decline in response to internal

420 challenges, such as infection, inflammation or stress (Murata et al., 2004). ALB has 421 been reported to be more highly expressed in the duodenum of helminth-naïve 422 genetically-resistant animals compared to susceptible individuals (Keane et al., 2006) 423 and to decline in the abomasum of sheep during the course of repeated truncated 424 immunising infections with Trichostrongylus colubriformis larvae (Knight et al., 425 2010). ALB levels were also increased in the abomasal mucosa of resistant selectionline animals compared to their susceptible counterparts 3 days after experimental 426 427 challenge with *Haemonchus contortus* (Nagaraj et al., 2012). In a separate study on 428 changes in abomasal protein expression following trickle infection with T. 429 circumcincta, ALB was relatively highly expressed in the mucosa of helminth-naïve 430 animals in comparison to both their immune and immune-waning counterparts 431 (Pemberton et al., 2012). In the same study serum albumin was significantly lower in 432 GIN immune sheep compared to naïve controls. Pemberton et al. hypothesised that 433 albumin may be constitutively released into the gastric mucus and may therefore play 434 an innate protective role. In this study, the response to GIN infection in the HighFEC 435 animals appears to be similar to that previously observed in naïve animals, suggesting 436 that the HighFEC animals may not be generating a timely immune response to GIN 437 infection. Expression of the Solute Carrier Family 30 (Zinc Transporter), Member 2 (SLC30A2) was also increased in HighFEC animals. SLC30A2 is involved in the 438 essential maintenance of cellular  $Zn^{2+}$  (Huang and Tepaamorndech, 2013). Studies 439 440 using zinc-deficient nematode-infected mouse models have shown that parasites are 441 better able to survive in zinc-deficient hosts compared to well-nourished hosts, with the function of T cells and antigen-presenting cells impaired by zinc deficiency (Scott 442 443 and Koski, 2000).

444 At 14 dpi, 6 genes were identified as DE between HighFEC and LowFEC animals based on tagwise dispersion. Of note is ECTL2 (epithelial cell transforming sequence 445 2 oncogene-like), expression of which was higher in LowFEC than HighFEC animals. 446 447 Recurrent somatic mutations in ECT2L have been associated with early T-cell precursor acute lymphoblastic leukaemia (Zhang et al., 2012). In contrast to the 448 449 findings from this study, expression of ECT2L has been reported to be increased in Suffolk (relatively susceptible) when compared to Texel (relatively resistant) lambs 450 451 over the course of a controlled challenge with T. circumcincta (Ahmed, 2013) and the 452 reason for these opposing results remains to be resolved. Expression of a putative 453 MHC class I antigen (ENSOARG0000001701) was also increased in LowFEC 454 animals. Class I antigens have previously been associated with reduced FEC in 455 Scottish Blackface lambs (Buitkamp et al., 1996; Stear et al., 1996a), and with genetic susceptibility in naïve sheep (Keane et al., 2006). 456

# 457 4.3 Temporal changes in gene expression in the abomasal lymph node of lambs458 challenged with T. circumcincta

459 In the HighFEC animals, expression of ALB and MX2 (Myxovirus resistance 2) were significantly higher at 7 dpi compared to 14 dpi. The down-regulation of ALB by 14 460 dpi was by far the most significant change, with a fold change of 166 (FDR =  $8.5 \times 10^{-10}$ 461 462 <sup>24</sup>). Expression of *MX2* is strongly induced by IFN- $\alpha$  (Kane et al., 2013; Melén et al., 463 1996), and the MX2 protein shows antiviral activity (Sasaki et al., 2014). Despite the 464 association between this gene and viral infections, it has previously been shown to be 465 increased at both the mRNA and protein levels in GIN susceptible animals (Ahmed, 466 2013; Nagaraj et al., 2012). The genes LYVE1 (Lymphatic vessel endothelial 467 hyaluronan receptor 1) and CHI3L2 (chitinase 3-like 2) were more highly expressed

468 in HighFEC animals at 14 compared to 7 dpi. LYVE1 is a major receptor for hvaluronan on the lymph vessel wall (Banerij et al., 1999). Hvaluronan is an abundant 469 470 component of skin and mesenchymal tissues, where it facilitates cell migration during 471 wound healing, inflammation, and embryonic morphogenesis. Chitinase-like proteins such as CHI3L2 also have a role in inflammation, tissue remodelling and injury (Lee 472 473 et al., 2011). Increased expression of CHI3L2 has been observed in the abomasum of 18 and 21 week old steers exposed to Ostertagia ostertagi and the abomasal lymph 474 node of resistant and susceptible Scottish Blackface lambs infected with T. 475 476 circumcincta in comparison to sham-infected controls (Gossner et al., 2013). In 477 human macrophages, CHI3L2 has been found to be up-regulated by IL-4 and TGF-B 478 (Gratchev et al., 2008). The increased expression of these genes indicates that 479 HighFEC animals are mounting an inflammatory response at approximately 14 dpi.

480 Like their HighFEC counterparts, LowFEC animals expressed higher levels of 481 MX2 at 7 dpi than at 14 dpi. While all of the DE genes that were increased in 482 LowFEC animals at 14 dpi are currently annotated as "novel protein coding" genes in 483 Ensembl (Ensembl Release 78), several are orthologous to Bos taurus and Homo sapiens MHC genes. ENSOARG0000016098 is a 1-to-1 ortholog of BOLA-DRB3. 484 485 ENSOARG0000002985 and ENSOARG00000015866 are one-to-many orthologs of 486 BOLA-DOA1 and BOLA-DOB, respectively. ENSOARG00000010572 is a many-to-487 many ortholog of multiple human MHC class I genes. The MHC is involved in the 488 induction and regulation of the immune response, and associations between the MHC 489 genes and both resistance and susceptibility to GIN have been found in multiple 490 studies reviewed by Venturina et al. (2013) (Venturina et al., 2013).

### 491 *4.4 Pathway analysis*

492 Biological processes involving 'inflammatory response', 'attraction of T lymphocytes' and 'binding of leucocytes' were more highly expressed in resistant 493 494 animals at 7 dpi. Therefore, these animals appear to be generating an earlier immune 495 response to infection than susceptible animals, through an increase in migration of 496 cells involved in the response to pathogens. Increased cellular recruitment to the 497 lymph node in resistant animals was also indicated by the increased weight of the 498 abomasal lymph node tissue. Upstream regulators of the genes involved in these 499 processes included TNF, IFN- $\alpha$ , IFN- $\beta$  and IFN- $\lambda$ . The pro-inflammatory cytokine 500 TNF is produced in the gastric mucosa during inflammation and can promote gastrointestinal homeostasis, although excess TNF production can contribute to 501 502 gastric mucosal inflammation and injury (Wallace and Ma, 2001), and has been 503 implicated in the intestinal pathology of nematode infections (Lawrence et al., 1998). 504 Following deliberate infection with T. circumcincta, elevated TNF expression has 505 been observed in both the abomasal lymph node of challenged compared to 506 unchallenged lambs at 5 days post infection (Craig et al., 2007), and in the abomasal 507 mucosa of DRB1\*1101 carrier lambs compared to their non-carrier counterparts at 3 508 days post infection (Hassan et al., 2011). The type I IFNs ( $\alpha$  and  $\beta$ ) can be produced 509 by almost every cell type, including leukocytes. While IFNs are best known for their 510 role in the cellular response to viral infections, they also possess immunomodulatory 511 activities (González-Navajas et al., 2012). The connection between type I IFNs and 512 several human autoimmune and inflammatory disorders is well known, with several 513 inflammatory syndromes shown to benefit from the administration of type I IFNs 514 (González-Navajas et al., 2012). While the antiviral capability of type III IFNs, 515 including IFN- $\lambda$ , is not as highly studied as that of the type I IFNs, they have their

516 own profile of immunomodulatory functions, specifically at the immune/epithelial517 interface (Gallagher et al., 2010).

Taken together, this indicates that the LowFEC animals are mounting a response that involves inflammatory cytokines, immune cell recruitment and the synthesis of reactive oxygen species by 7 dpi. By approximately 14 dpi, genes downstream from these regulators were no longer up-regulated in the LowFEC animals; however, they were activated in the HighFEC animals. This indicates that the susceptible animals may have a delayed immune response to infection compared to resistant lambs.

### 525 4.5 Comparison with other studies

526 Intestinal smooth muscle contractility (Diez-Tascon et al., 2005), pathogen 527 recognition, via MHC class II molecules, and T helper cell polarisation (Gill et al., 2000; Hassan et al., 2011; Hein et al., 2004) have all been previously suggested as 528 529 processes that control the response to GIN (Keane et al., 2007; Pernthaner et al., 530 2005). Of these, T helper cell polarisation has probably the most support (Hassan et al., 2011; Muñoz-Guzmán et al., 2012; Pemberton et al., 2011). However, these 531 532 pathways were primarily identified in studies that compared animals that differed in 533 worm burden. Worm burden may influence gene expression directly and some of the 534 biological processes detected may therefore be a consequence of the differences in 535 worm burden rather than the cause. We examined gene expression in a breed that 536 primarily manifests resistance by regulating worm fecundity, although regulation of 537 worm burden may also contribute (McRae et al., 2014; Stear et al., 1995; Stear et al., 538 1996b). Despite the differences between the present study and previous studies, many 539 of the DE genes observed in this study were previously reported in other studies

540 examining resistance to GIN in sheep via regulation of worm burden, including those investigating the transcriptome (Ahmed, 2013; Gossner et al., 2013) and the proteome 541 542 (Nagaraj et al., 2012). Only 1 DE gene (IL13) was common with Salle et al (2014), a 543 study which examined gene expression differences in lambs that controlled worm 544 fecundity, although this may be due to the fact that they examined a limited number of 545 genes, at a different time-point, and in lambs infected with a different parasite species (Sallé et al., 2014). A systems genetics study based on data from multiple QTL and 546 547 gene expression studies, led to the identification of common pathways between genes 548 in QTL associated with genetic resistance to GIN in various populations (Sayre and 549 Harris, 2012). This suggests that, despite the difference mechanisms of GIN resistance 550 between breeds, there may be some common pathways associated with GIN resistance 551 across breeds of sheep and GIN species.

552 The number of genes identified as DE using tagwise dispersion estimates was 553 relatively low when compared to other RNA-Seq studies of the same tissue (Ahmed, 554 2013; Gossner et al., 2013; Pemberton et al., 2011). However, it must be noted that 555 the methods for identifying DE genes varied between the studies. Gossner et al. used the Limma package, which was developed and optimised for array data, while Ahmed 556 used EdgeR with common dispersion estimates. When using common dispersion 557 558 estimates in this study, the number of DE genes was similar to that reported by 559 Ahmed. Another possibility is that there may be variation among animals in how they 560 manifest resistance and the timing of manifestation. In this scenario common dispersion may be a more appropriate technique for identifying differential 561 562 expression. Indeed, a larger number of significantly DE genes were found using this 563 method.

### 564 4.6 SNP analysis

565 We hypothesised that markers in DE genes may be associated with resistance. The 566 goal was not to identify causative mutations *per se* but to test markers which may be 567 in linkage disequilibrium with causative mutations. As DE genes detected by tagwise dispersion were more consistent between animals within a group, markers in these 568 569 genes were tested for association with FEC. While a number of SNP in ALB and 570 SLC30A2 were suggestively associated with FEC2<sub>OT</sub> they were not significant after 571 correction for multiple testing. The present study is limited by the number of animals 572 (237) available for association analysis. Previous studies have involved between 752 573 and 1275 individuals (Riggio et al., 2013; Sallé et al., 2012) for GWAS. Future work 574 on validating the SNP of interest from this study would require a larger number of 575 Scottish Blackface animals. As noted in reports on multiple studies, host resistance to 576 GIN appears to be mediated by many genes, each with a relatively small effect 577 (Kemper et al., 2011; Riggio et al., 2014). The candidate gene approach is therefore 578 unlikely to capture all of the variation underlying known phenotypic differences; 579 future work on identifying SNP to be used in selection programmes will likely focus 580 on utilising a panel of SNP in addition to searching for individual causative mutations. 581 Despite these limitations, suggestive associations between FEC2<sub>OT</sub> and multiple SNP 582 within the same gene indicate that genotyping SNP within DE genes may be a valid 583 way of discovering polymorphisms associated with GIN resistance.

## 584 **5. Conclusions**

585 Transcriptional profiling of the abomasal lymph node during a controlled challenge 586 with *T. circumcincta* indicated that in resistant (LowFEC) Scottish Blackface lambs, 587 pathways relating to the inflammatory response, migration of T lymphocytes and

588 synthesis of reactive oxygen species were more highly expressed at 7 dpi. In their 589 susceptible (HighFEC) counterparts this response was delayed until ~14 dpi 590 indicating that resistant animals are generating an earlier immune response to *T*. 591 *circumcincta*. SNP in 2 DE genes (*SLC30A2* and *ALB*), were suggestively associated 592 with FEC.

# 594 **Conflict of Interest Statement**

595 The authors declare that they have no conflict of interests.

## 597 Acknowledgements

598 The authors gratefully acknowledge the technical assistance of Assumpta Glynn, 599 Margaret Murray, Joe Larkin, Kevin Fitzsimmons, Simone Sebastiano, Luke O'Malley P.J. Hastings and Henry Walsh. Special thanks to Tom McNeilly from the 600 601 Moredun Research Institute for supplying the T. circumcincta L3 antigen, and 602 Rudiger Brauning and John McEwan from AgResearch for supplying the flanking sequences for the Ovine Infinium® HD SNP BeadChip SNP. Kathryn McRae was 603 604 supported by a Teagasc Walsh fellowship (grant number 6001) and the Allan and 605 Grace Kay Overseas Scholarship.

## 626 **Figures**

## 627 **Figure 1 – Experimental design.**

Purebred Scottish Blackface lambs were used in the study. Flock faecal count (FEC) 628 629 was monitored weekly from when lambs were approximately 8 weeks of age. Once 630 this reached ~600 eggs per gram (epg) lambs were individually sampled twice, 1 631 week apart, to give FEC1. Lambs were then dosed with an anthelmintic, and returned to pasture where the process was repeated for the second natural infection (FEC2). 632 633 Blood was collected from all animals for DNA extraction. FEC1 and FEC2 values for 634 other trichostrongyles from the cohort of 92 2010-born male Scottish Blackface lambs<sup>a</sup> were used to select the 10 most resistant (LowFEC) and 10 most susceptible 635 (HighFEC) lambs for gene expression studies. Phenotypic measurement was repeated 636 in 2011 on both male and female  $lambs^{b}$  (n = 166), resulting in FEC1 and FEC2 637 measurements from 258 lambs<sup>a,b</sup>, which were used for the genetic association study. 638

639

- Figure 2 Response of susceptible (HighFEC) and resistant (LowFEC) lambs
   to natural and artificial challenge GIN infection.
- 643 Mean ( $\pm$ s.e) faecal egg count of HighFEC (n = 10) and LowFEC (n = 10) individuals
- 644 during natural challenge (A). Mean (±s.e) circulating basophil numbers (B), Mean
- 645 (±s.e) serum anti-*Teladorsagia circumcincta* IgA (C) and Mean (±s.e) mucosa anti-*T*.
- 646 circumcincta IgA (D) in HighFEC and LowFEC animals during a controlled
- 647 challenge with 3 x  $10^4$  *T. circumcincta* L3 larvae.
- 648

## 649 Figure 3 - Top 20 DE diseases and biological functions.

- 650 Heat map of the top 20 IPA-derived diseases and biological functions from genes DE
- between HighFEC and LowFEC animals at 7 or 14 dpi, sorted by P value.

## 653 **Figure 4 - Top 10 IPA-derived upstream regulators.**

- 654 Heat map of the top 10 IPA-derived upstream regulators from genes DE between
- HighFEC and LowFEC animals at 7 or 14 dpi, sorted by P value.

## 657 **References**

658	Abdi, H., 2007, The Bonferonni and Šidák Corrections for Multiple Comparisons, In:
659	Salkind, N.J. (Ed.) Encyclopedia of Measurement and Statistics. SAGE
660	Publications Inc, Thousand Oaks, CA.
661	Ahmed, A.M., 2013. Immunological and transcriptomic analysis of genetically
662	resistant and susceptible sheep to gastrointestinal nematodes. Doctoral Thesis.
663	University College Dublin, Dublin, Ireland.
664	Allen, J.E., Maizels, R.M., 2011, Diversity and dialogue in immunity to helminths.
665	Nature Reviews Immunology 11, 375-388.
666	Andronicos, N.M., Hunt, P., Windon, R., 2010, Expression of genes in
667	gastrointestinal and lymphatic tissues during parasite infection in sheep
668	genetically resistant or susceptible to Trichostrongylus colubriformis and
669	Haemonchus contortus. International Journal for Parasitology 40, 417-429.
670	Anon, 1986, Modified McMaster Method. In Ministry of Agriculture Fisheries and
671	Food: Manual of Veterinary Parasitological Laboratory Techniques.
672	(Reference book 418) London.
673	Banerji, S., Ni, J., Wang, SX., Clasper, S., Su, J., Tammi, R., Jones, M., Jackson,
674	D.G., 1999, LYVE-1, a New Homologue of the CD44 Glycoprotein, Is a
675	Lymph-specific Receptor for Hyaluronan. The Journal of Cell Biology 144,
676	789-801.
677	Benjamini, Y., Hochberg, Y., 1995, Controlling the False Discovery Rate: A Practical
678	and Powerful Approach to Multiple Testing. Journal of the Royal Statistical
679	Society. Series B Methodological 57, 289-300.
680	Biomart, http://www.ensembl.org/biomart/martview.
681	Bishop, S.C., Morris, C.A., 2007, Genetics of disease resistance in sheep and goats.
682	Small Ruminant Research 70, 48-59.
683	Buitkamp, J., Filmether, P., Stear, M.J., Epplen, J.T., 1996, Class I and class II major
684	histocompatibility complex alleles are associated with faecal egg counts
685	following natural, predominantly Ostertagia circumcincta infection.
686	Parasitology Research 82, 693-696.
687	Craig, N.M., Miller, H.R.P., Smith, W.D., Knight, P.A., 2007, Cytokine expression in
688	naïve and previously infected lambs after challenge with Teladorsagia
689	circumcincta. Veterinary Immunology and Immunopathology 120, 47-54.
690	Culhane, A.C., Thioulouse, J., Perrière, G., Higgins, D.G., 2005, MADE4: an R
691	package for multivariate analysis of gene expression data. Bioinformatics 21,
692	2789-2790.
693	Diez-Tascon, C., Keane, O.M., Wilson, T., Zadissa, A., Hyndman, D.L., McEwan,
694	J.C., Crawford, A.M., 2005, Microarray analysis of selection lines from
695	outbred populations to identify genes involved with nematode parasite
696	resistance in sheep. Physiological Genomics 21, 59-69.
697	Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P.,
698	Chaisson, M., Gingeras, T.R., 2013, STAR: ultrafast universal RNA-seq
699	aligner. Bioinformatics 29, 15-21.
700	ENCODE,
701	http://genome.ucsc.edu/ENCODE/protocols/dataStandards/ENCODE_RNAse
702	<u>q_Standards_V1.0.pdf</u> .
703	Fecpak, <u>http://www.techiongroup.co.nz/products/fecpak</u> .

704	Gallagher, G., Megjugorac, N.J., Yu, R.Y., Eskdale, J., Gallagher, G.E., Siegel, R.,
705	Tollar, E., 2010, The Lambda Interferons: Guardians of the Immune-
706	Epithelial Interface and the T-helper 2 Response. Journal of Interferon &
707	Cytokine Research 30, 603-615.
708	Gill, H.S., Altmann, K., Cross, M.L., Husband, A.J., 2000, Induction of T helper 1-
709	and T helper 2-type immune responses during Haemonchus contortus
710	infection in sheep. Immunology 99, 458-463.
711	Gilmour, A.R., Gogel, B.J., Cullis, B.R., Thompson, R. 2009. ASReml User Guide
712	Release 3.0 (Hemel Hempstead, HP1 1ES, UK, VSN International Ltd), pp. 1-
713	398.
714	González-Navajas, J.M., Lee, J., David, M., Raz, E., 2012, Immunomodulatory
715	functions of type I interferons. Nature Reviews Immunology 12, 125-135.
716	Gossner, A.G., Wilkie, H., Joshi, A., Hopkins, J., 2013, Exploring the abomasal
717	lymph node transcriptome for genes associated with resistance to the sheep
718	nematode <i>Teladorsagia circumcincta</i> . Veterinary Research 44, 68.
719	Gratchev, A., Schmuttermaier, C., Mamidi, S., Gooi, L., Goerdt, S., Kzhyshkowska,
720	J., 2008, Expression of Osteoarthritis Marker YKL-39 is Stimulated by
721	Transforming Growth Factor Beta (TGF-beta) and IL-4 in Differentiating
722	Macrophages. Biomarker Insights 3, 39-44.
723	Hassan, M., Hanrahan, J.P., Good, B., Mulcahy, G., Sweeney, T., 2011, A differential
724	interplay between the expression of Th1/Th2/Treg related cytokine genes in
725	Teladorsagia circumcincta infected DRB1*1101 carrier lambs. Veterinary
726	Research 42, 45.
727	Hein, W.R., Barber, T., Cole, SA., Morrison, L., Pernthaner, A., 2004, Long-term
728	collection and characterization of afferent lymph from the ovine small
729	intestine. Journal of Immunological Methods 293, 153-168.
730	HTSeq, http://www-huber.embl.de/users/anders.HTSeq.
731	Huang, L., Tepaamorndech, S., 2013, The SLC30 family of zinc transporters – A
732	review of current understanding of their biological and pathophysiological
733	roles. Molecular Aspects of Medicine 34, 548-560.
734	Jiang, Y., Xie, M., Chen, W., Talbot, R.T., Maddox, J.F., Faraut, T., Wu, C., Muzny,
735	D.M., Li, Y., Zhang, W., Stanton, JA., Brauning, R., Barris, W.C., Hourlier,
736	T., Aken, B.L., Searle, S.M.J., Adelson, D.L., Bian, C., Cam, G.R., Chen, Y.,
737	Cheng, S., DeSilva, U., Dixen, K., Dong, Y., Fan, G., Franklin, I.R., Fu, S.,
738	Fuentes-Utrilla, P., Guan, R., Highland, M.A., Holder, M.E., Huang, G.,
739	Ingham, A.B., Jhangiani, S.N., Kalra, D., Kovar, C.L., Lee, S.L., Liu, W., Liu,
740	X., Lu, C., Lv, T., Mathew, T., McWilliam, S., Menzies, M., Pan, S., Robelin,
741	D., Servin, B., Townley, D., Wang, W., Wei, B., White, S.N., Yang, X., Ye,
742	C., Yue, Y., Zeng, P., Zhou, Q., Hansen, J.B., Kristiansen, K., Gibbs, R.A.,
743	Flicek, P., Warkup, C.C., Jones, H.E., Oddy, V.H., Nicholas, F.W., McEwan,
744	J.C., Kijas, J.W., Wang, J., Worley, K.C., Archibald, A.L., Cockett, N., Xu,
745	X., Wang, W., Dalrymple, B.P., 2014, The sheep genome illuminates biology
746	of the rumen and lipid metabolism. Science 344, 1168-1173.
747	Kane, M., Yadav, S.S., Bitzegeio, J., Kutluay, S.B., Zang, T., Wilson, S.J., Schoggins,
748	J.W., Rice, C.M., Yamashita, M., Hatziioannou, T., Bieniasz, P.D., 2013,
749	MX2 is an interferon-induced inhibitor of HIV-1 infection. Nature 502, 563-
750	566.
751	Karasuyama, H., Mukai, K., Obata, K., Tsujimura, Y., Wada, T., 2011, Nonredundant
752	Roles of Basophils in Immunity. Annual Review of Immunology 29, 45-69.

753	Keane, O.M., Dodds, K.G., Crawford, A.M., McEwan, J.C., 2007, Transcriptional
754	profiling of Ovis aries identifies Ovar-DQA1 allele frequency differences
755	between nematode-resistant and susceptible selection lines. Physiological
756	Genomics 30, 253-261.
757	Keane, O.M., Zadissa, A., Wilson, T., Hyndman, D.L., Greer, G.J., McCulloch, A.F.,
758	Crawford, A.M., McEwan, J.C., 2006, Gene expression profiling of naïve
759	sheep genetically resistant and susceptible to gastrointestinal nematodes. BMC
760	Genomics 7, 42.
761	Kemper, K.E., Elwin, R.L., Bishop, S.C., Goddard, M.E., Woolaston, R.R., 2009,
762	Haemonchus contortus and Trichostrongylus colubriformis did not adapt to
763	long-term exposure to sheep that were genetically resistant or susceptible to
764	nematode infections. International Journal for Parasitology 39, 607-614.
765	Kemper, K.E., Emery, D.L., Bishop, S.C., Oddy, V.H., Hayes, B.J., Dominik, S.,
766	Henshall, J.M., Goddard, M.E., 2011, The distribution of SNP marker effects
767	for faecal worm egg count in sheep, and the feasibility of using these markers
768	to predict genetic merit for resistance to worm infections. Genetics Research
769	93, 203-219.
770	Knight, J., Hein, W., Pernthaner, A., 2010, The gastrointestinal nematode
771	Trichostrongylus colubriformis down-regulates immune gene expression in
772	migratory cells in afferent lymph. BMC Immunology 11, 51.
773	Knight, P.A., Griffith, S.E., Pate, J.M., Guarneri, L., Anderson, K., Talbot, R.T.,
774	Smith, S., Waddington, D., Fell, M., Archibald, A.L., Burgess, S.T.G., Smith,
775	D.W., Miller, H.R.P., Morrison, I.W., 2011, Novel gene expression responses
776	in the ovine abomasal mucosa to infection with the gastric nematode
777	<i>Teladorsagia circumcincta</i> . Veterinary Research 42, 78.
778	Kumar, P., Henikoff, S., Ng, P.C., 2009, Predicting the effects of coding non-
779	synonymous variants on protein function using the SIFT algorithm. Nature
780	Protocols 4, $10/3-1081$ .
/81	Lawrence, C.E., Paterson, J.C., Higgins, L.M., MacDonald, T.T., Kennedy, M.W.,
782 782	Garside, P., 1998, IL-4-regulated enteropathy in an intestinal nematode
105	Lee C.C. De Silve C.A. Dele Cruz C.S. Abengeri F. Me P. Keng M. I. He C.
70 <del>4</del> 785	H. Takyar S. Elias, I.A. 2011 Bole of chitin and chitinase/chitinase like
786	notains in inflammation, tissue remodeling, and injury. Annual Peview of
780	Physiology 73, 479, 501
788	MacKinnon KM Burton LL Zajac A M Notter D.R. 2000 Microarray analysis
789	reveals difference in gene expression profiles of hair and wool sheep infected
790	with Haemonchus contortus. Veterinary Immunology and Immunonathology
791	130 210-220
792	McRae K M Good B Hanrahan I P Glynn A O'Connell M I Keane O M
793	2014 Response to <i>Teladorsagia circumcincta</i> infection in Scottish Blackface
794	lambs with divergent phenotypes for nematode resistance. Veterinary
795	Parasitology 206, 200-207.
796	Melén, K., Keskinen, P., Ronni, T., Sareneva, T., Lounatmaa, K., Julkunen, I., 1996.
797	Human MxB Protein, an Interferon- $\alpha$ -inducible GTPase. Contains a Nuclear
798	Targeting Signal and Is Localized in the Heterochromatin Region beneath the
799	Nuclear Envelope. Journal of Biological Chemistry 271, 23478-23486.
800	Montgomery, G.W., Sise, J.A., 1990, Extraction of DNA from sheep white blood
801	cells. New Zealand Journal of Agricultural Research 33, 437-441.
	-

802	Muñoz-Guzmán, M.A., Cuenca-Verde, C., Valdivia-Anda, G., Cuéllar-Ordaz, J.A.,
803	Alba-Hurtado, F., 2012, Differential immune response between fundic and
804	pyloric abomasal regions upon experimental ovine infection with <i>Haemonchus</i>
805	contortus. Veterinary Parasitology 185, 175-180.
806	Murata, H., Shimada, N., Yoshioka, M., 2004, Current research on acute phase
807	proteins in veterinary diagnosis: an overview. The Veterinary Journal 168, 28-
808	40.
809	Nagaraj, S.H., Harsha, H.C., Reverter, A., Colgrave, M.L., Sharma, R., Andronicos,
810	N.M., Hunt, P., Menzies, M., Lees, M.S., Sekhar, N.R., Pandey, A., Ingham,
811	A., 2012, Proteomic analysis of the abomasal mucosal response following
812	infection by the nematode, Haemonchus contortus, in genetically resistant and
813	susceptible sheep. Journal of Proteomics 75, 2141-2152.
814	Pemberton, A.D., Brown, J.K., Craig, N.M., Pate, J.M., McLean, K., Inglis, N.F.,
815	Knox, D., Knight, P.A., 2012, Changes in protein expression in the sheep
816	abomasum following trickle infection with <i>Teladorsagia circumcincta</i> .
817	Parasitology 139, 375-385.
818	Pemberton, J.M., Beraldi, D., Craig, B.H., Hopkins, J., 2011, Digital gene expression
819	analysis of gastrointestinal helminth resistance in Scottish blackface lambs.
820	Molecular Ecology 20, 910-919.
821	Pernthaner, A., Cole, S.A., Morrison, L., Hein, W.R., 2005, Increased Expression of
822	Interleukin-5 (IL-5), IL-13, and Tumor Necrosis Factor Alpha Genes in
823	Intestinal Lymph Cells of Sheep Selected for Enhanced Resistance to
824	Nematodes during Infection with Trichostrongylus colubriformis. Infection
825	and Immunity 73, 2175-2183.
826	Riggio, V., Matika, O., Pong-Wong, R., Stear, M.J., Bishop, S.C., 2013, Genome-
827	wide association and regional heritability mapping to identify loci underlying
828	variation in nematode resistance and body weight in Scottish Blackface lambs.
829	Heredity 110, 420-429.
830	Riggio, V., Pong-Wong, R., Sallé, G., Usai, M.G., Casu, S., Moreno, C.R., Matika,
831	O., Bishop, S.C., 2014, A joint analysis to identify loci underlying variation in
832	nematode resistance in three European sheep populations. Journal of Animal
833	Breeding and Genetics, 1-11.
834	Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010, edgeR: a Bioconductor package
835	for differential expression analysis of digital gene expression data.
836	Bioinformatics 26, 139-140.
837	Robinson, M.D., Oshlack, A., 2010, A scaling normalization method for differential
838	expression analysis of RNA-seq data. Genome Biology 11, R25.
839	Rowe, A., Gondro, C., Emery, D., Sangster, N., 2009, Sequential microarray to
840	identify timing of molecular responses to Haemonchus contortus infection in
841	sheep. Veterinary Parasitology 161, 76-87.
842	Safari, E., Fogarty, N.M., Gilmour, A.R., 2005, A review of genetic parameter
843	estimates for wool, growth, meat and reproduction traits in sheep. Livestock
844	Production Science 92, 271-289.
845	Sallé, G., Jacquiet, P., Gruner, L., Cortet, J., Sauvé, C., Prevot, F., Grisez, C.,
846	Bergeaud, J.P., Schibler, L., Tircazes, A., Francois, D., Pery, C., Bouvier, F.,
847	Thouly, J.C., Brunel, J.C., Legarra, A., Elsen, J.M., Bouix, J., Rupp, R.,
848	Moreno, C.R., 2012, A genome scan for QTL affecting resistance to
849	Haemonchus contortus in sheep. Journal of Animal Science 90, 4690-4705.

850	Sallé, G., Moreno, C., Boitard, S., Ruesche, J., Tircazes-Secula, A., Bouvier, F.,
851	Aletru, M., Weisbecker, JL., Prevot, F., Bergeaud, J.P., Trumel, C., Grisez,
852	C., Liénard, E., Jacquiet, P., 2014, Functional investigation of a QTL affecting
853	resistance to Haemonchus contortus in sheep. Veterinary Research 45, 68.
854	Sasaki, K., Tungtrakoolsub, P., Morozumi, T., Uenishi, H., Kawahara, M., Watanabe,
855	T., 2014, A single nucleotide polymorphism of porcine MX2 gene provides
856	antiviral activity against vesicular stomatitis virus. Immunogenetics 66, 25-32.
857	Sayre, B.L., Harris, G.C., 2012, Systems genetics approach reveals candidate genes
858	for parasite resistance from quantitative trait loci studies in agricultural
859	species. Animal Genetics 43, 190-198.
860	Scott, M.E., Koski, K.G., 2000, Zinc Deficiency Impairs Immune Responses against
861	Parasitic Nematode Infections at Intestinal and Systemic Sites. The Journal of
862	Nutrition 130, 1412S-1420S.
863	Stear, M.J., Bairden, K., Bishop, S.C., Buitkamp, J., Epplen, J.T., Gostomski, D.,
864	McKellar, Q.A., Schwaiger, F.W., Wallace, D.S., 1996a, An ovine
865	lymphocyte antigen is associated with reduced faecal egg counts in four-
866	month-old lambs following natural, predominantly Ostertagia circumcincta
867	infection. International Journal for Parasitology 26, 423-428.
868	Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes, P.H., Irvine, J.,
869	McCririe, L., McKellar, Q.A., Sinski, E., Murray, M.A.X., 1995, Regulation
870	of egg production, worm burden, worm length and worm fecundity by host
871	responses in sheep infected with Ostertagia circumcincta. Parasite
872	Immunology 17, 643-652.
873	Stear, M.J., Park, M., Bishop, S.C., 1996b, The key components of resistance to
874	Ostertagia circumcincta in lambs. Parasitology Today 12, 438-441.
875	TrimGalore, http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/.
876	Venturina, V.M., Gossner, A.G., Hopkins, J., 2013, The immunology and genetics of
877	resistance of sheep to Teladorsagia circumcincta. Veterinary Research
878	Communications 37, 171-181.
879	Wallace, J.L., Ma, L., 2001, Inflammatory mediators in gastrointestinal defense and
880	injury. Experimental biology and medicine (Maywood, N.J.) 226, 1003-1015.
881	Zaros, L.G., Neves, M.R.M., Benvenuti, C.L., Navarro, A.M.C., Sider, L.H.,
882	Coutinho, L.L., Vieira, L.S., 2014, Response of resistant and susceptible
883	Brazilian Somalis crossbreed sheep naturally infected by Haemonchus
884	contortus. Parasitology Research 113, 1155-1161.
885	Zhang, J., Ding, L., Holmfeldt, L., Wu, G., Heatley, S.L., Payne-Turner, D., Easton,
886	J., Chen, X., Wang, J., Rusch, M., Lu, C., Chen, SC., Wei, L., Collins-
887	Underwood, J.R., Ma, J., Roberts, K.G., Pounds, S.B., Ulyanov, A., Becksfort,
888	J., Gupta, P., Huether, R., Kriwacki, R.W., Parker, M., McGoldrick, D.J.,
889	Zhao, D., Alford, D., Espy, S., Bobba, K.C., Song, G., Pei, D., Cheng, C.,
890	Roberts, S., Barbato, M.I., Campana, D., Coustan-Smith, E., Shurtleff, S.A.,
891	Raimondi, S.C., Kleppe, M., Cools, J., Shimano, K.A., Hermiston, M.L.,
892	Doulatov, S., Eppert, K., Laurenti, E., Notta, F., Dick, J.E., Basso, G., Hunger,
893	S.P., Loh, M.L., Devidas, M., Wood, B., Winter, S., Dunsmore, K.P., Fulton,
894	R.S., Fulton, L.L., Hong, X., Harris, C.C., Dooling, D.J., Ochoa, K., Johnson,
895	K.J., Obenauer, J.C., Evans, W.E., Pui, CH., Naeve, C.W., Ley, T.J., Mardis,
896	E.R., Wilson, R.K., Downing, J.R., Mullighan, C.G., 2012, The genetic basis
897	of early T-cell precursor acute lymphoblastic leukaemia. Nature 481, 157-163.
898	

Comparison		Common dispersion	Tagwise dispersion
		<i>FDR</i> <0.05	<i>FDR</i> <0.1
HighFEC vs LowFEC	7 dpi	194	7
	14 dpi	144	6
7 vs 14 dpi	HighFEC	224	7
•	LowFEC	163	14

# Table 1 – Number of genes differentially expressed using common and tagwise dispersion analyses

FDR = False discovery rate.

Comparison	Up in	Ensembl ID	Gene	Description	<b>FC</b> <sup>1</sup>	CPM <sup>2</sup>	FDR <sup>3</sup>
7 dpi	HighFEC	ENSOARG00000013782	ALB	Albumin		1.7	< 0.01
		ENSOARG00000020224	COL9A2	Collagen, type IX, alpha 2	6.4	17.1	0.01
		ENSOARG00000011275	-	Uncharacterized protein	4.2	1.7	0.01
		ENSOARG0000003000	-	Uncharacterized protein	0.6	12.5	0.01
		ENSOARG0000005312	-	-	0.7	35.2	0.01
		ENSOARG0000001778	OSBPL5	Oxysterol binding protein-like 5	0.4	2.1	0.02
		ENSOARG0000005490	SLC30A2	Solute carrier family 30 (zinc transporter), member 2	0.5	46.1	0.03
14 dpi	HighFEC	ENSOARG0000020373	MFI2	Antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5	3.7	19.3	< 0.01
		ENSOARG00000011529	ZFR2	Zinc finger RNA binding protein 2	2.5	99.6	0.05
	LowFEC	ENSOARG0000005549	-	40S ribosomal protein S3a	2.9	9.7	< 0.01
		ENSOARG0000001701	-	Uncharacterized protein	2.1	1136.4	< 0.01
		ENSOARG0000004858	-	Uncharacterized protein	3.7	6.6	0.04
		ENSOARG0000000928	ECT2L	Epithelial cell transforming 2 like	0.2	166.9	0.04
HighFEC	7 dpi	ENSOARG00000013782	ALB	Albumin	0.2	202.5	< 0.01
		ENSOARG00000010231	MX2	Myxovirus (influenza virus) resistance 2 (mouse)	0.4	59.5	0.04
		ENSOARG0000004858	-	Uncharacterized protein	0.3	74.8	0.08
	14 dpi	ENSOARG00000010111	-	Uncharacterized protein	0.4	1.7	0.03
		ENSOARG00000011072	LYVE1	Lymphatic vessel endothelial hyaluronan receptor 1	0.3	28.1	0.07
		ENSOARG00000019641	LSAMP	Limbic system-associated membrane protein	0.7	151.9	0.08
		ENSOARG00000019517	CHI3L2	Chitinase 3-like 2	0.5	38.4	0.08
LowFEC	7 dpi	ENSOARG00000010231	MX2	Myxovirus (influenza virus) resistance 2 (mouse)	0.5	8.2	< 0.01
		ENSOARG00000020801	-	Uncharacterized protein	0.2	1.9	0.02
		ENSOARG00000020811	-	Uncharacterized protein	0.4	3.3	0.02
		ENSOARG00000020789	-	Uncharacterized protein	0.1	3.8	0.03
		ENSOARG00000020194	MAB21L3	Mab-21-like 3 (C. elegans)	0.4	5.4	0.04
	14 dpi	ENSOARG00000016098	-	Uncharacterized protein	0.6	12.8	< 0.01
		ENSOARG0000002985	-	Uncharacterized protein	0.4	5.2	0.02
		ENSOARG00000010572	-	Uncharacterized protein	0.4	2.1	0.03
		ENSOARG00000020792	-	Uncharacterized protein	0.3	2.0	0.03
		ENSOARG0000001720	-	Uncharacterized protein	0.3	4.4	0.03

## Table 2 – Genes identified as differentially expressed in abomasal lymph node using tagwise dispersion

ENSOARG0000000058	-	Uncharacterized protein	4.7	63.6	0.03
ENSOARG00000015866	-	Uncharacterized protein	1.8	352.1	0.03
ENSOARG0000001279	-	Galectin-14 (LOC443162)	3.6	1.6	0.09
ENSOARG0000005126	-	Uncharacterized protein	5.2	3.6	0.10

 $^{1}FC = fold$ -change.  $^{2}CPM = counts$ -per-million.  $^{3}FDR = false$  discovery rate.

Comparison	Network	IPA Score <sup>1</sup>	Genes
7 dpi	Cell-To-Cell Signaling and Interaction, Cellular Movement, Immune Cell Trafficking	32	17
HighFEC vs LowFEC	Amino Acid Metabolism, Cardiovascular Disease, Hematological Disease	25	14
	Developmental Disorder, Hereditary Disorder, Metabolic Disease	25	14
	Cardiovascular System Development and Function, Organismal Development, Visual System Development and Function	22	13
	Energy Production, Cellular Development, Connective Tissue Development and Function	20	12
14 dpi	Cellular Assembly and Organization, Lipid Metabolism, Small Molecule Biochemistry	41	19
HighFEC vs LowFEC	Cell Signaling, Molecular Transport, Nucleic Acid Metabolism	23	12
	Gene Expression, Organ Morphology, Cell Morphology	20	11
	Developmental Disorder, Endocrine System Disorders, Organismal Injury and Abnormalities	16	9
	Hematological Disease, Respiratory Disease, Hematological System Development and Function	16	9
HighFEC	Cancer, Cellular Movement, Connective Tissue Development and Function	45	23
7 vs 14 dpi	Carbohydrate Metabolism, Small Molecule Biochemistry, Energy Production	35	19
	Developmental Disorder, Drug Metabolism, Energy Production	35	19
	Infectious Disease, Inflammatory Disease, Neurological Disease	30	17
	Cellular Compromise, Hereditary Disorder, Skeletal and Muscular Disorders	21	13
LowFEC	Cardiovascular System Development and Function, Tissue Development, Organismal Development	33	17
7 vs 14 dpi	Cell-To-Cell Signaling and Interaction, Cellular Movement, Immune Cell Trafficking	28	15
	Connective Tissue Disorders, Hereditary Disorder, Metabolic Disease	24	13
	Neurological Disease, Developmental Disorder, Endocrine System Disorders	24	13
	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	24	13

## Table 3 – Top 5 networks for each comparison identified by Ingenuity Pathway Analysis using DE genes.

<sup>1</sup>IPA network score is expressed as the -log(Fisher's exact test p-value).

Comparison	Ingenuity Canonical Pathways	<b>BH P value</b> <sup>1</sup>	Ratio <sup>2</sup>	Genes
7 dpi	Pathogenesis of Multiple Sclerosis	0.00002	0.44	CXCL10, CXCL11, CCR5, CXCL9
HighFEC vs LowFEC <sup>3</sup>	Hepatic Fibrosis / Hepatic Stellate Cell Activation	0.01	0.04	CCR5, COL13A1, LAMA1, LBP, CXCL9, COL9A2, MMP9
LowFEC	Pathogenesis of Multiple Sclerosis	0.000007	0.44	CXCL10, CXCL11, CCL5, CXCL9
7 vs 14 dpi <sup>4</sup>	IL-17A Signaling in Gastric Cells	0.01	0.12	CXCL10, CXCL11, CCL5
	Tryptophan Degradation to 2-amino-3-	0.02	0.25	<i>KMO</i> , <b>IDO</b> 1
	carboxymuconate Semialdehyde			
	Granulocyte Adhesion and Diapedesis	0.04	0.03	CXCL10, CXCL11, CCL22, CCL5, CXCL9
	Agranulocyte Adhesion and Diapedesis	0.04	0.03	CXCL10, CXCL11, CCL22, CCL5, CXCL9
	NAD biosynthesis II (from tryptophan)	0.04	0.13	<i>KMO</i> , <i>IDO1</i>

Table 4 – Significant canonical pathways identified by Ingenuity Pathway Analysis using differentially expressed (DE) genes.

<sup>1</sup>BH P value = Benjamini-Hochberg corrected P value. <sup>2</sup>The ratio is calculated by taking the number of DE genes that participate in a Canonical

Pathway, and dividing it by the total number of genes in that Canonical Pathway. <sup>3</sup>Genes more highly expressed in LowFEC animals are in bold, while genes more highly expressed in HighFEC animals are normal typeface. <sup>4</sup>Genes more highly expressed at 7 dpi are in bold, while genes more highly expressed at 14 dpi are normal typeface.

Table 5 – Association between SNP in differentially expressed genes and gastrointestinal nematode resistance traits in Scottish Blackface lambs

				2			_		-	P value <sup>8</sup>			
Ensembl gene ID	Name	OAR	Position	SNP ID <sup>2</sup>	Consequence	SIFT <sup>4</sup>	MAF	Alleles <sup>®</sup>	n'	FEC1	FEC1	$FEC2_O$	$FEC2_N$
										OT	NEM	Т	EM
ENSOARG0000020224	COL9A2	1	14688520	rs425322992	Missense	0.45	0.38	C/T	237	0.67	0.63	0.40	0.45
		1	14691551	rs402057753	N/A	-	0.38	G/A	237	0.67	0.63	0.40	0.45
ENSOARG0000020373	MFI2	1	189728511	rs405258217	N/A	-	0.11	T/C	237	0.40	0.08	0.77	0.75
		1	189741476	rs424822608	N/A	-	0.21	A/G	237	0.99	0.99	0.39	0.83
ENSOARG0000005490	SLC30A2	2	239929582	rs408045395	Missense	1	0.27	G/T	237	0.97	0.93	0.03	0.35
		2	239931409	rs414806237	Missense	0	0.03	T/A	237	0.64	0.48	0.03	0.47
		2	239932238	rs426472558	Synonymous	-	0.47	T/C	237	0.97	0.38	0.25	0.84
ENSOARG00000011529	ZFR2	5	17723806	rs414694841	Missense	1	0.46	G/A	237	0.87	0.44	0.50	0.20
		5	17725481	rs406633004	Missense	0.81	0.08	G/A	237	0.67	0.21	0.69	0.90
		5	17734892	rs409768412	N/A	-	0.45	A/A	237	0.90	0.62	0.41	0.27
ENSOARG00000013782	ALB	6	88140673	rs399322137	N/A	-	0.39	G/A	237	0.23	0.58	0.03	0.70
		6	88146314	rs412988422	Missense	0.9	0.27	A/C	237	0.45	0.81	0.79	0.99
		6	88151153	rs398193652	N/A	-	0.16	G/T	235	0.30	0.08	0.03	0.59
ENSOARG0000000928	ECT2L	8	63609947	rs427698166	N/A	-	0.12	C/A	236	0.22	0.40	0.19	0.96
		8	63613802	rs402663145	N/A	-	0.12	G/A	237	0.20	0.43	0.24	0.94
		8	63636645	rs160248153	Missense	0.1	0.17	G/T	236	0.41	0.29	0.61	0.75
		8	63639731	rs411594326	Missense	1	0.17	C/T	237	0.42	0.35	0.58	0.73
ENSOARG0000005312	-	14	46056292	rs429181932	Missense	0.36	0.15	G/A	237	0.47	0.30	0.47	0.15
		14	46070690	rs160946903	Synonymous	-	0.28	C/A	236	0.84	0.81	0.30	0.34
		14	46070771	rs162105426	Missense	0.81	0.25	C/T	237	0.08	0.25	0.18	0.41
ENSOARG0000005549	-	17	6550163	rs405054724	N/A	-	0.17	A/G	237	0.28	0.99	0.82	0.48
ENSOARG00000001778	OSBPL5	21	47759083	rs422189173	N/A	-	0.48	T/C	237	0.16	0.48	0.81	0.11
		21	47759570	rs411124861	N/A	-	0.48	T/C	237	0.13	0.29	0.97	0.06
		21	47792907	rs161640197	Synonymous	-	0.22	G/A	237	0.72	0.58	0.53	0.18

<sup>1</sup>Chromosome, <sup>2</sup>SNP = dbSNP (release 140) <sup>3</sup>Consequence = consequence of variant on the protein sequence, N/A indicates variant is intronic. <sup>4</sup>SIFT = effect of substitution on protein function: green indicates change is predicted to be tolerated, red indicates change is predicted to be deleterious. <sup>5</sup>MAF = minor allele frequency. <sup>6</sup>Alleles = first allele/second allele (second allele is the minor allele). <sup>7</sup>n = number of animals with genotypes. <sup>8</sup>P-value = uncorrected significance value for each variable. After Bonferroni correction the significance level thresholds were P <  $2.08 \times 10^{-3}$  and P < 4.17 x 10<sup>-2</sup> for genome-wide significance (P < 0.05) and suggestive significance (that is, one false positive per genome scan), respectively. Suggestive associations SNP are highlighted in bold.



Infection on pasture

Controlled challenge







### Figure 3

# ACCEPTED MANUSCR

↑ in LowFEC ↑ in LowFEC ↑ in HighFEC ↑ in HighFEC Melanoma Inflammatory response Malignant solid tumour Migration of cardiac fibroblasts Abdominal cancer Digestive tract cancer Malignant neoplasm of digestive system Obesity Synthesis of reactive oxygen species Attraction of T lymphocytes Neoplasia of epithelial tissue Attraction of Th0 lymphocytes Attraction of plasmacytoid dendritic cells Liver cancer Homeostasis of lipid Storage of lipid Atherogenic dyslipidemia Insulin resistance Epithelial cancer Binding of leukocytes



