Transcriptional changes in *Teladorsagia circumcincta* upon encountering host tissue of differing immune status

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SUMMARY

The aim of this study was to elucidate transcriptional changes in the parasitic nematode *Teladorsagia circumcincta* upon encountering either naïve or immune ovine hosts. Pools of 100 000 exsheathed 3rd- stage *T. circumcincta* larvae were exposed *in vitro* to either an immune or naïve ovine abomasal environment, RNA was extracted from the larvae and sequenced using the Roche 454 platform. Each sample produced approximately 82 000 reads that assembled to give approximately 5500 Isotigs (contigs). The two sequence datasets were clustered together to give a total of 6969 clusters of which 18 were differentially expressed ($P<0{\cdot}001$) between the two groups. Clusters with a predominance of reads in larvae exposed to the immune abomasal environment encoded homologues of peptidyl-glycine alpha-amidating monooxygenase, heat shock-protein 16-2 and IDA-1, a tyrosine phosphatase-like receptor protein. Clusters with a predominance of reads in the naïve environment encoded homologues of cytochrome b, EGg Laying defective family member 21 and NADH dehydrogenase subunit 5. Gene ontology analyses indicated that larvae exposed to the immune environment showed an increase in expression of genes involved in 'carbon utilization', 'response to stimulus' and 'developmental process'. These data suggest that *T. circumcincta* modulates gene expression in response to the immune status of the host.

Key words: *Teladorsagia circumcincta*, gene expression, parasitic nematodes, sheep, proteomics, immunity.

INTRODUCTION

Teladorsagia circumcincta is a parasitic nematode that inhabits the abomasum of small domestic ruminants. It is an important parasite in temperate areas of the world, both in terms of animal welfare and economic loss. Animals suffer from diarrhoea, reduced growth, emaciation and high morbidity; the estimated cost to the British farming industry through gastrointestinal parasites alone is £84 million annually (Nieuhof and Bishop, 2005; Scott *et al*. 2007). Current control methods rely on pasture management and the use of anthelmintic drugs; however, the emergence of widespread resistance to anthelmintic treatment is driving the search for alternative methods of control (Sargison, 2011).

Sheep can acquire immunity to *T. circumcincta* following exposure, leading to investigation of vaccination as one such alternative method of control (Smith *et al*. 1983, 1986; Miller, 1996; Balic *et al*. 2003; Macaldowie *et al*. 2003; Halliday *et al*. 2009, 2010). Manifestations of immunity include a reduction in total worm burden and egg output, a reduction in developing worm length and a reduction in larval establishment in the abomasa of immune sheep, through either exclusion or expulsion, within 48 h of ingestion (Stear *et al*. 1995, 1999*a*, *b*; Jackson *et al*. 2004; Halliday *et al*. 2007, 2009). This implies that there are critical events occurring very early in the host-parasite interaction that determine whether the parasite can establish in the abomasum of its ovine host.

The high capacity and relatively long read length of 454 sequencing technology (Margulies *et al*. 2005; Roche, 2011) has made it suitable for transcriptomic studies and several parasite species have been investigated in this way including *T. circumcincta*, *Haemonchus contortus*, *Trichostrongylus colubriformus* and *Fasciola hepatica* (Young *et al*. 2003; Cantacessi *et al*. 2010*a*, *b*; Dicker *et al*. 2011). Here, we used 454 sequencing to examine gene expression in the infective 3rd larval stage of *T. circumcincta* immediately following exposure to either an immune or a naïve ovine host abomasal environment. By comparing gene expression in these environments we have identified a subset of the genes that may be important in larval infectivity and response to host immunity.

MATERIALS AND METHODS

Animals, post-mortem and generation of naïve and immune abomasal environments

All sheep used were Scotch Mules (Blackface ewe X Blue-faced Leicester tup) that were 8 months old at

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the start of the immunizing infection, and 10 months old at post-mortem. The sheep were born and raised in controlled parasite-free conditions to ensure that they remained parasite free until the time of trickle infection for the immunized group, and until the end of the experiment for the naïve group. Immunity to *T. circumcincta* was generated in 1 group of 6 sheep as detailed previously (Halliday *et al*. 2007). Briefly, a trickle infection of 2000 infective 3rd-stage larvae (L3) *T. circumcincta* was given 3 times per week for 8 weeks. This group of animals (immune) were then given Fenbendazole according to the manufacturer's instructions to remove the resident worms, followed 7 days later, by a single bolus challenge of 50 000 L3. Another group of 6 uninfected sheep (naïve) were maintained in parallel. Twenty-four hours after the bolus dose of larvae to the immune group, all the sheep were stunned with a captive bolt, exsanguinated, and the abomasum removed and opened along its lesser curvature. The abomasum was washed 3 times in warm saline, then two 2 cm^2 folds per sheep were removed, rinsed briefly with warm water to remove food debris and then incubated in 2 ml of warm Earle's Buffered Saline Solution (18 mM $CaCl_2 \cdot 2H_2O$, 54 mM KCl, 8 mM $MgSO_4 \cdot 7H_2O$, 1·16 M NaCl, 10 mM NaH_2PO_4, 56 mM glucose; EBSS) for 3 h at 37 °C, in 6-well cell culture plates. The abomasal fold sample was then removed and discarded, and the buffer retained as either a naive or immune abomasal environment sample.

Ex-sheathing larvae and exposure to abomasal environment

Approximately 200 000 *T. circumcincta* L3 were washed in phosphate-buffered saline (PBS)/penicillin (250 IU/ml)/streptomycin (250 mg/ml)/gentamicin (312·5 μg/ml)/amphotericin B (5 μg/ml) (PBS wash solution), then centrifuged for 2 min at 200 ***g*** and the supernatant fraction discarded. This was repeated twice, then the larval pellet was re-suspended in 50 ml of warm EBSS/penicillin (100 IU/ml)/streptomycin (100 mg/ml)/gentamicin (125 μg/ml/amphotericin b (5 μg/ml) (EBSS ex-sheathing buffer). CO_2 gas was bubbled gently through the larval suspension for 15 min, before incubating the suspension at 37 °C with shaking (100 rpm) for 4 h. Then 100 μl was removed every hour to monitor larval exsheathing. After 4 h of incubation, the larval suspension was split into 2 equal volumes and centrifuged for 2 min at 200 ***g***. The supernatant fraction was removed and the larval pellets were resuspended in 1·5 ml of naïve or immune abomasal environment sample, prepared as described above, then incubated in 6-well plates at 37 °C with shaking (100 rpm) for a further 4 h. A 100 μl sample was removed after 2 h and 4 h to monitor larval viability. After 4 h the larvae were centrifuged for 2 min at 200 ***g***. The supernatant fraction was removed, and 1 larval pellet for each treatment snap frozen in liquid nitrogen.

Preparation of cDNA and 454 sequencing

Total RNA from the 2 larval samples, i.e. 1 from naïve- and 1 from immune-abomasal environment exposed larvae was prepared using TRIzol reagent (GibcoBRL, Life Technologies) following the manufacturer's instructions. The extracted RNA was treated with DNase (Ambion) as per the manufacturer's instructions, and cleaned up using a Qiagen RNeasy Minelute kit, as per manufacturer's instructions. RNA integrity and yield was examined by agarose gel electrophoresis and using a Nano-Drop ND-1000 UV spectrophotometer v.3.2.1 (NanoDrop Technologies, Wilmington, DE, USA). First strand cDNA was constructed using the SMARTer™ PCR cDNA synthesis kit (Clontech/Takara Bio, CA) following the manufacturer's instructions. An optimized PCR cycling protocol of 14 cycles was used to amplify full-length cDNAs using 5′ PCR Primer II A with Advantage 2 PCR buffer and polymerase mix (Clontech/Takara), and the amplified cDNA cleaned up using Qiagen PCR purification kit as per manufacturer's instructions. A total of 5 μg cDNA for each sample was delivered to TheGenePool, Edinburgh University, for next generation sequencing using Roche-454 Titanium technology (Margulies *et al*. 2005; Roche, 2011).

Bioinformatic analysis of sequencing data

Sequence data were assembled using Newbler (v2.55) Assembler software (Roche, 2010). The read data from each population were assembled separately, as joint assembly of the 2 sets of sequencing data resulted in unrealistically long contigs that were unlikely to represent biological entities. Following assembly, the Isotig sequences representing putative transcripts were submitted to the Blast2GO server (Conesa *et al*. 2005; Conesa and Gotz, 2008; Gotz *et al*. 2008). This carried out Blastx searches using the NCBI non-redundant database (Blast ExpectValue 1.0E-03), InterProScan searches for functional annotation (Hunter *et al*. 2009) and Gene Ontology based annotation (Ashburner *et al*. 2000). Sequences were annotated to KEGG biological pathways within Blast2GO with the parameters E-Value-Hit-Filter 1.0E-06, Annotation CutOff 55, GO weight 5 (Kanehisa and Goto, 2000; Ogata *et al*. 1999). Clustering of the combined Isotig datasets was carried out using UCLUST (v3.0.617) (Edgar, 2010) optimized at 65% identity, searching against forward and reverse strand, with optimal global alignment selected. The reads contained within each cluster were enumerated using a Perl script

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that calculated the total number of unique reads originating from each sample and thus the abundance of transcripts from each sample.

Statistical analyses

A 2 × 2 Fisher's Exact test was conducted to test for differences between the mean proportion of Isotigs representing naïve and immune groups for each functional classification as identified by KEGG and GO analysis. The estimated *P*-values for all functional groups under KEGG and GO classification systems were then adjusted using the False Discovery Rate (FDR) approach (Benjamini and Hochberg, 1995) to take into account multiple comparisons of treatments.

For the 454 next-generation sequencing data, the strategy used to identify clusters indicated that there was likely to be excess clustering or overdispersion in the data. However, with a single replicate for each combination of cluster and treatment, it was not possible to quantify this effect. Therefore an approach was adopted which combined information from 2 statistical models that together spanned the range of results that would arise from a more appropriate model. The first model is a conservative model with dispersion parameter set at a maximum possible value (3·65), estimated from a separate model which confounded any cluster or cluster by treatment effects with this dispersion parameter. The second model is a liberal model which assumes that there is no overdispersion in the data, and hence sets the value of dispersion parameter as 1. Details of this statistical approach were previously described by Dicker *et al*. (2011).

The FDR adjusted *p*-value (Benjamini and Yekutieli, 2005) for a cluster is the minimum FDR for which the observed difference between the mean expression level of naïve and immune groups would be accepted as statistically significant. The FDR-adjusted *P* values (P_F) from the two statistical models were then used to categorize each cluster into 1 of 3 classes: clusters with P_F values of less than or equal to 5% under the conservative model were of prime scientific interest; clusters with P_F values greater than 5% under the liberal model were unlikely to be of interest; other clusters were of indeterminate status, but could be prioritized for further work with respect to either set of P_F values. FDR-adjusted *p*-values are hereafter denoted by P_F.

All statistical analyses were carried out using the R software version 2.11.1 (R Development Core Team, 2010).

Proteomic analysis of abomasal environments

SDS-PAGE of naive and immune abomasal environment samples was carried out using NuPAGE 4–12% Bis-Tris mini gels (Invitrogen, Life Technologies, Carlsbad, CA, USA) under reducing conditions, as per manufacturer's instructions. After protein separation, gels were stained with colloidal Coomassie Blue (SimplyBlue™ SafeStain, Invitrogen) then destained in distilled water. Mass spectrometry analysis was carried out at the Moredun Research Institute's Proteomics Facility as described previously (Moredun, 2011; Smith *et al*. 2009). Mascot generic (mgf) files were generated from the resultant data and submitted to a local database server using the MASCOT search engine for protein database searching against NCBI non-redundant database (http://www.ncbi.nlm.nih.gov) with mammals as a taxonomical search parameter. The fixed and variable modifications used in the searches were carbamidomethyl (C) and oxidation (M) respectively. Mass tolerance values for MS and MS/MS were set at 1·5 Da and 0·5 Da, respectively. Matches achieving a significant molecular weight search (MOWSE) score were considered significant only if 2 or more peptides were matched for each protein, and each was observed to contain an unbroken '*b*' or '*y*' ion series of a minimum of 4 amino acid residues.

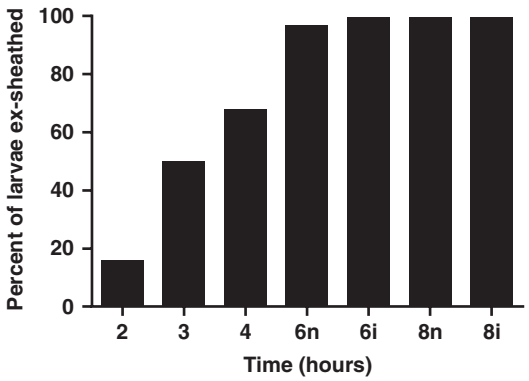


Fig. 1. Progress of larval exsheathing. 6-n and 8-n, larvae incubated in naïve abomasal environment sample. 6-i and 8-i, larvae incubated in immune abomasal environment sample.

RESULTS

Ex-sheathing larvae

The percentage of ex-sheathed larvae at each time-point sampled during the ex-sheathing process and during the exposure to the abomasal environment samples are shown in Fig. 1. After incubation for 2 h in EBSS ex-sheathing buffer, 15% of the larvae had ex-sheathed. After 3 h, 49% had ex-sheathed and after 4 h 67% had ex-sheathed. At each of these time-points no larvae had died (data not shown). After incubation for 4 h in ex-sheathing buffer the larvae were transferred to incubation in either the immune or naive abomasal environment samples. The numbers of larvae that had ex-sheathed after 2 h and 4 h in this

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Table 1. Overview of bioinformatic analysis

(a, Isotigs which share 1 or more 454 reads are grouped by the Newbler assembler into 'Isogroups', so that the Isotigs within 1 Isogroup could represent the alternative isoforms from the same gene.)

	Immune exposed	Naïve exposed
Raw data and assembly information		
Number of reads	82 096	82 267
Number assembling into Isotigs	59 240 (72%)	63 825 (78%)
Number of Isotigs	5525	5395
Number of Isogroups[a]	4651	4617
Homology searches(Using Isotig consensus sequences)		
With homology to sequences in NCBI non-redundant database	3736 (68%)	3550 (66%)
With predicted gene ontology (GO) terms	2651 (48%)	2560 (47%)
Returning InterProScan results	2733 (49%)	3078 (57%)
Returning enzyme match	848 (15%)	724 (13%)
Biological pathways predicted (KEGG)	104	98
Total number of Clusters (combined dataset)	6969	

environment are represented by the 6-n and 8-n bars (incubated in naive environment sample) and the 6-i and 8-i bars (incubated in immune environment sample) in Fig. 1. After incubation for 8 h, more than 99% of the larvae were ex-sheathed and alive.

Bioinformatic analysis

An overview of the sequencing data, assembly and initial bioinformatic analysis is shown in Table 1. The number of Isotigs in each dataset with Gene Ontology annotations, InterProScan results and enzyme matches, and the total number of Clusters obtained after combining the 2 sets of Isotigs, are also shown.

Gene ontology

The level 2 Gene Ontology terms associated with the Isotigs from each sample are shown in Table 2. Table 3 shows the 'biological process' and 'cellular component' GO terms that had a significantly higher mean proportion of Isotigs in the immune exposed group than the naïve exposed group. For biological processes these included 'carbon utilization', 'response to stimulus' and 'developmental process' as exemplified by CRE-ALDO-2 protein (fructose-bisphosphate aldolase 2, 44 Isotigs), heat shock protein 16–2 (68 Isotigs) and heat shock protein 16–2 (68 Isotigs)/CRE-GEI-7 protein (isocitrate lyase-like protein, 65 Isotigs) respectively (all $P_F < 0{\cdot}001$). Within 'cellular component', mean proportion of Isotig for 'membrane-enclosed lumen' and 'macromolecular complex' were statistically significantly higher in the immune-exposed group compared to the naïve-exposed group ($P_F \leqslant 0{\cdot}001$). There was no evidence that the number of any level 2 'molecular function' annotations were statistically different between the exposed and naïve group.

KEGG biological pathways

KEGG biological pathway annotation of the 2 sets of Isotigs was carried out within the Blast2GO software suite and pathways showing statistically significant differences in the mean proportion of Isotigs between the two groups are shown in Table 4. Pathways showing statistically significantly higher mean proportion of Isotigs in the larvae exposed to the immune environment ($P_F \leqslant 0{\cdot}001$) were 'carbohydrate metabolism', 'biosynthesis of alkaloids', 'biosynthesis of phenylpropanoids, plant hormones, terpenoids and steroids', 'biosynthesis of secondary metabolites' and 'metabolic pathways'. Immune exposed larvae also showed a statistically significantly higher number of Isotigs for metabolic pathways ($P_F = 0{\cdot}039$).

Most abundant transcripts

Clustering of combined sets of 5525 Isotigs from the immune exposed larval sample and the 5395 Isotigs from the naïve-exposed sample using UCLUST software resulted in 6969 Clusters. The 10 most abundant sequences for each sample, determined by the number of unique reads contributing to the Cluster, are shown in Table 5. In the sample that had been prepared from larvae exposed to the immune abomasal environment, the most abundant sequences were a homologue of a hypothetical protein homologue from *Brugia malayi*, an unidentified protein that had no hits in the NCBI non-redundant database, and a senescence-associated protein also from *B. malayi*. The most abundant sequences in the naïve-exposed larval sample were the same hypothetical protein homologue from *B. malayi* identified in the immune-exposed larvae, Cytochrome b and NADH dehydrogenase subunit 5.

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Table 2. Parental (level 2) gene ontology (GO) terms associated with the assembled Isotigs from each sample

(GO categories were assigned within the Blast2GO software according to hits from the NCBI non-redundant database, and also according to InterPro domains with homology to functionally annotated genes.)

	Number of Isotigs (%)	
	Immune exposed	Naïve exposed
Biological Process (GO:0008150)	*2164 (39·2)*	*2088 (38·7)*
cellular process (GO:0009987)	1524 (27·6)	1320 (24·5)
metabolic process (GO:0008152)	1246 (22·6)	1220 (22·6)
multicellular organismal process (GO:0032501)	1299 (23·5)	1070 (19·8)
developmental process (GO:0032502)	1244 (22·5)	996 (18·5)
biological regulation (GO:0065007)	1026 (18·6)	838 (15·5)
growth (GO:0040007)	860 (15·6)	658 (12·2)
reproduction (GO:0000003)	723 (13·1)	627 (11·6)
localization (GO:0051179)	599 (10·8)	583 (10·8)
locomotion (GO:0040011)	440 (8·0)	375 (7·0)
cellular component organization (GO:0016043)	365 (6·6)	360 (6·7)
response to stimulus (GO:0050896)	416 (7·5)	255 (4·7)
signaling (GO:0023052)	289 (5·2)	250 (4·6)
cellular component biogenesis (GO:0044085)	114 (2·1)	74 (1·4)
death (GO:0016265)	39 (0·7)	31 (0·6)
biological adhesion (GO:0022610)	13 (0·2)	20 (0·4)
multi-organism process (GO:0051704)	10 (0·2)	17 (0·3)
rhythmic process (GO:0048511)	4 (0·1)	4 (0·1)
cell proliferation (GO:0008283)	5 (0·1)	4 (0·1)
cell wall organization or biogenesis (GO:0071554)	5 (0·1)	3 (0·1)
nitrogen utilization (GO:0019740)	1 (0·0)	2 (0·0)
carbon utilization (GO:0015976)	58 (1·0)	2 (0·0)
pigmentation (GO:0043473)	0 (0·0)	1 (0·0)
immune system process (GO:0002376)	3 (0·1)	1 (0·0)
viral reproduction (GO:0016032)	0 (0·0)	1 (0·0)
cell killing (GO:0001906)	1 (0·0)	0 (0·0)
Cellular Component (GO:0005575)	*1350 (24·4)*	*1297 (24·0)*
cell (GO:0005623)	1289 (23·3)	1210 (22·4)
organelle (GO:0043226)	604 (10·9)	598 (11·1)
macromolecular complex (GO:0032991)	397 (7·2)	281 (5·2)
extracellular region (GO:0005576)	97 (1·8)	99 (1·8)
membrane-enclosed lumen (GO:0031974)	91 (1·6)	31 (0·6)
synapse (GO:0045202)	29 (0·5)	26 (0·5)
Molecular function (GO:0003674)	*2128 (38·5)*	*2114 (39·2)*
binding (GO:0005488)	1534 (27·8)	1523 (28·2)
catalytic activity (GO:0003824)	1225 (22·2)	1139 (21·1)
transporter activity (GO:0005215)	194 (3·5)	155 (2·9)
structural molecule activity (GO:0005198)	99 (1·8)	118 (2·2)
molecular transducer activity (GO:0060089)	69 (1·2)	70 (1·3)
electron carrier activity (GO:0009055)	47 (0·9)	68 (1·3)
enzyme regulator activity (GO:0030234)	51 (0·9)	64 (1·2)
transcription regulator activity (GO:0030528)	64 (1·2)	56 (1·0)
antioxidant activity (GO:0016209)	15 (0·3)	14 (0·3)
metallochaperone activity (GO:0016530)	1 (0·0)	1 (0·0)
channel regulator activity (GO:0016247)	2 (0·0)	0 (0·0)

Differences in sequence abundance between the larval samples

Individual sequencing reads contributing to each Cluster were identified, and parsed to output the number of unique reads per cluster originating from each sample. Analysis of these Cluster read counts resulted in 18 Clusters showing statistical significance at levels consistent with a false discovery rate of less than 5% ($P_F < 0{\cdot}05$) for differences in mean proportions of reads between the naïve and immune groups under the conservative model, while a further 77 Clusters were of indeterminate status, being statistically significant with $P_F < 0{\cdot}05$ under the liberal model only. The 18 Clusters with $P_F \leqslant 0{\cdot}05$ under the conservative model and the identity of the representative sequence of each (determined by Blastx homology searching to proteins in the NCBI non-redundant database) are shown in Table 6. A total of 11 Clusters had statistically significantly

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Table 3. GO terms with significantly more abundant representation within the immune exposed larval

	Number of Isotigs (%)		*P*
	Immune exposed	Naïve exposed	(FDR adjusted)
Biological process			
carbon utilization	58 (1·0)	2 (0·0)	<0·001
response to stimulus	416 (7·5)	255 (4·7)	<0·001
developmental process	1244 (22·5)	996 (18·4)	<0·001
growth	860 (15·5)	658 (12·2)	<0·001
multicellular organismal process	1299 (23·5)	1070 (19·8)	<0·001
biological regulation	1026 (18·5)	838 (15·5)	<0·001
cellular process	1524 (27·5)	1320 (24·4)	<0·001
cellular component biogenesis	114 (2·1)	74 (1·4)	0·020
Cellular component			
membrane-enclosed lumen	91 (1·6)	31 (0·6)	<0·001
macromolecular complex	397 (7·2)	281 (5·2)	<0·001

Table 4. KEGG annotations showing significant differences in abundance between the immune and naïve exposed larvae

	Number of sequences (%)		*P*
Pathway category	Immune exposed	Naïve exposed	(FDR adjusted)
Carbohydrate metabolism	481 (8·7)	216 (4·0)	<0·001
Biosynthesis of alkaloids	349 (6·3)	155 (2·9)	<0·001
Biosynthesis of phenylpropanoids, plant hormones, terpenoids and steroids	273 (4·9)	120 (2·2)	<0·001
Biosynthesis of secondary metabolites	151 (2·7)	71 (1·3)	<0·001
Metabolic pathways	367 (6·6)	293 (5·4)	0·039

higher mean proportion of reads in the immune-exposed larvae than naïve larvae ($P_F \leqslant 0{\cdot}001$). These included Cluster 295 (CBR-PGAL-1 protein – a peptidylglycine alpha-amidating mono-oxygenase homologue), Cluster 520 (heat shock protein 16-2), Cluster 273 (NADH dehydrogenase subunit 4) and Cluster 2240 (CRE-IDA-1 protein, a tyrosine phosphatase-like receptor protein). A total of 7 Clusters had a statistically significantly greater mean proportion of reads in the naïve larval sample ($P_F \leqslant 0{\cdot}001$) which included Cluster 334 (cytochrome b), Cluster 108 (EGg Laying defective family member (EGL-21) and Cluster 538 (NADH dehydrogenase subunit 5). Four of the 11 Clusters with $P_F \leqslant 0{\cdot}05$ and a higher mean proportion of reads originating from the immune exposed larvae, and 3 of the 7 Clusters with $P_F \leqslant 0{\cdot}05$ and a higher mean proportion of reads originating from the naïve-exposed larvae, were also amongst the 10 Clusters with the highest read counts for their respective originating samples. Furthermore, 8 of the 11 Clusters with $P_F \leqslant 0{\cdot}05$ for the immune-exposed larvae appeared to be unique to that dataset, with no representative reads from the naïve-exposed larval group.

In the set of indeterminate Clusters with $P_F \leqslant 0{\cdot}05$ only under the liberal model (Table 7), the immune-exposed group had a statistically significantly higher mean proportion of reads for sequences (with top Blastx hits) of CRE-EFT-2 (translation elongation factor 2), *Brachionus plicatilis* LEA-like protein 1 (late embryo abundant), the *B. malayi* senescence-associated protein and *T. circumcincta* GTP-Cyclohydrolase. The indeterminate clusters that showed a statistically significantly higher mean proportion of read counts in naïve-exposed larvae relative to immune-exposed larvae returned sequences (top Blastx hits) of *Caenorhabditis briggsae* UNC-54 protein (myosin heavy chain), *C. briggsae* putative acyltransferase, *Oesophagostomum dentatum* FMRFamide-like propeptide (*flp-14*), *Caenorhabditis elegans* UBC-18 homologue (ubiquitin-conjugating enzyme) and *Caenorhabditis remanei* EPN-1 protein (nucleoside transporter protein).

Proteomics results

Analysis of the proteins present in the naïve and immune abomasal environment samples returned 702 and 695 matches respectively in the NCBInr database. After *in silico* subtraction and manual curation to remove matches with the same identity, this was reduced to 67 protein matches uniquely detected in the naive abomasal environment, and

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Table 5. Top 10 most abundant sequences in each dataset, by number of reads from sample present in cluster

(a, Cluster appears in top 10 most abundant in both samples; -, no hits in NCBInr database. b, Blastx search using representative sequence as query.)

Cluster	Number of reads	Length of rep. seq. (bp)	Best alignment in NCBI non-redundant database[b]			
			Species	Identity	Accession no.	*e*-value
Immune exposed larvae						
Cluster 15[a]	3479	2561	*Brugia malayi*	Hypothetical protein Bm_11025	EDP37487.1	6E-23
Cluster 611	1739	947	—	—	—	—
Cluster 2[a]	1308	4021	*Brugia malayi*	Senescence-associated protein	EDP31077.1	5E-57
Cluster 6	957	3036	*Caenorhabditis remanei*	CRE-EFT-2 protein	EFP00955.1	0.0
Cluster 95[a]	813	1715	*Caenorhabditis elegans*	C-term-of-AEX-Binding protein family member (cab- 1)	AAG17881.1	2E-62
Cluster 968[a]	790	781	*Teladorsagia circumcincta*	Cytochrome c oxidase subunit ii	ACX85202.1	1E-110
Cluster 290[a]	708	1235	*Caenorhabditis elegans*	hypothetical protein Y71G12B.4	AAK73930.1	8E-121
Cluster 26	666	2328	*Haemonchus contortus*	Phosphoenolpyruvate carboxykinase	AAA29180.1	0.0
Cluster 137[a]	664	1582	*T. circumcincta*	NADH dehydrogenase subunit 5	ACX85204.1	0.0
Cluster 295	664	1228	*Caenorhabditis briggsae*	CBR-PGAL-1 protein	CAP37494.1	2E-101
Naïve exposed larvae						
Cluster 15[a]	2653	2561	*Brugia malayi*	Hypothetical protein Bm_11025	EDP37487.1	6E-23
Cluster 334	1861	1173	*Teladorsagia circumcincta*	Cytochrome b	ACX85210.1	3E-143
Cluster 137[a]	1279	1582	*Teladorsagia circumcincta*	NADH dehydrogenase subunit 5	ACX85204.1	0.0
Cluster 135	1276	1584	*Teladorsagia circumcincta*	NADH dehydrogenase subunit 1	ACX85207.1	6E-112
Cluster 2[a]	1078	4021	*Brugia malayi*	Senescence-associated protein	EDP31077.1	5E-57
Cluster 968[a]	946	781	*Teladorsagia circumcincta*	Cytochrome c oxidase subunit ii	ACX85202.1	1E-110
Cluster 475	909	1047	*Caenorhabditis elegans*	C-type LECtin family member (clec-1)	AAB37085.1	1E-50
Cluster 95[a]	749	1715	*Caenorhabditis elegans*	C-term-of-AEX-Binding protein family member (cab- 1)	AAG17881.1	2E-62
Cluster 1225	721	704	—	—	—	—
Cluster 290[a]	704	1235	*Caenorhabditis elegans*	hypothetical protein Y71G12B.4	AAK73930.1	8E-121

67 protein matches uniquely detected in the immune abomasal environment sample (Table 8). Proteins identified uniquely in the immune sample included clathrin, intelectin-2, galectin-15, IgE and gelsolin-b.

DISCUSSION

In this study, we have presented data comparing the transcriptome of exsheathed infective *Teladorsagia circumcincta* 3rd larval stage parasites upon exposure to either a naïve or immune host environment, and also carried out qualitative proteomic analysis on the host environment samples. These data showed changes in transcript levels between the 2 larval populations, particularly for several genes with possible roles in sensory capabilities and response to the differing environments, in this case possibly reflecting early responses to the host immune response. The proteome profiles of the naïve and immune abomasal environments confirmed that the larvae exposed to the immune environment encountered relevant immune effectors.

A conservative statistical model was used, due to the lack of experimental repeats, to determine statistically significant differences in transcript abundance between the transcriptomes of the 2 larval samples. Eighteen transcripts were identified although, as a conservative statistical model was employed, the true number of transcripts with significantly differing abundance may be higher than this. O'Meara *et al*. (2010) also observed that changes in expression occurred in only a relatively small proportion of *Strongyloides ratti* transcripts in response to host immune status.

Analysis of the GOs associated with the entire transcriptome from each sample (i.e. not restricted to only those transcripts with higher mean abundance in the immune-exposed larvae) showed that in the immune-environment exposed larval sample the most statistically significantly increased annotations were 'carbon utilization', 'response to stimulus' and 'developmental process'. The increased number of transcripts with these annotations is likely to reflect the response of larvae encountering a different environment from those exposed to a naïve host environment.

One of the transcripts with the greatest increase in abundance in larvae exposed to the immune host environment was a *C. briggsae* peptidyl-glycine alpha-amidating monooxygenase protein homologue (CBR-PGAL-1 protein). The peptidyl-glycine

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Table 6. Identities of Clusters with statistically significant differences in mean read count abundance between immune-exposed and naïve-exposed groups under the conservative model

(a, cluster also amongst the top 10 most abundant for that sample. b, Blastx search using representative sequence as query. -, no hits in NCBInr database.)

	Read counts			Best alignment in NCBI non-redundant database[b]			
Cluster	Immune exposed	Naïve exposed	*P* (FDR adjusted)	Species	Identity	Accession	*e*-value
Clusters containing significantly more reads originating from immune exposed larvae							
Cluster 295[a]	664	0	<0·001	*Caenorhabditis briggsae*	CBR-PGAL-1 protein	CAP37494.1	1.9E-101
Cluster 520	446	0	<0·001	*Caenorhabditis elegans*	Heat shock protein 16-2	AAA28071.1	3.6E-21
Cluster 273	417	0	<0·001	*Teladorsagia circumcincta*	NADH dehydrogenase subunit 4	ACX85212.1	3.3E-96
Cluster 2240	147	0	<0·001	*Caenorhabditis remanei*	CRE-IDA-1 protein	EFO86750.1	1.3E-15
Cluster 242	298	44	<0·001	*Caenorhabditis remanei*	CRE-ALDO-2 protein	EFO86555.1	8.8E-163
Cluster 26[a]	666	293	<0·001	*Haemonchus contortus*	Phosphoenolpyruvate carboxykinase	AAA29180.1	0.0
Cluster 15[a]	3479	2653	<0·001	*Brugia malayi*	Hypothetical protein Bm_11025	EDP37487.1	6.2E-23
Cluster 611[a]	1739	0	0·010	—	—	—	—
Cluster 3523	48	0	0·017	—	—	—	—
Cluster 994	45	0	0·029	—	– (IPS matches: COIL, PTHR23241, seg.)	—	—
Cluster 6794	43	0	0·038	—	—	—	—
Clusters containing significantly more reads originating from naive exposed larvae							
Cluster 334[a]	158	1861	<0·001	*Teladorsagia circumcincta*	Cytochrome b	ACX85210.1	3.5E-143
Cluster 108	32	449	<0·001	*Caenorhabditis elegans*	EGg Laying defective family member (egl-21)	CAB02881.1	0.0
Cluster 538	0	532	<0·001	*Teladorsagia circumcincta*	NADH dehydrogenase subunit 5	ACX85204.1	4.1E-62
Cluster 1091	338	686	<0·001	*Teladorsagia circumcincta*	cytochrome c oxidase subunit 1	ACX85201.1	8.8E-110
Cluster 475[a]	2	909	0·003	*Caenorhabditis elegans*	C-type LECtin family member (clec-1)	AAB37085.1	1.3E-50
Cluster 135[a]	0	1276	0·010	*Teladorsagia circumcincta*	NADH dehydrogenase subunit 1	ACX85207.1	7.6E-112
Cluster 2749	11	116	0·038	—	– (IPS match: TMHMM)	—	—

alpha-amidating monooxygenase gene encodes 1 polypeptide with 2 enzymatic domains, responsible for the copper-dependent alpha-amidation of glycine-extended biopeptides often necessary for full activity of the peptides (Eipper *et al*. 1993; Prigge *et al*. 2000). Neuropeptides are commonly modified at the N- or C-terminus to protect them from degradation and/or to achieve full biological activity. Amidated peptides have widespread functions in the nervous system, and in development and regulation: in *C*. *elegans* over 18 genes encode more than 53 Phe-Met-Arg-Phe-NH_2 (FMRFamide) related peptides, yet disruption of a single precursor gene, and the resultant varying levels of the peptide, leads to a profound variation in behaviour (Nelson *et al*. 1998). In *Schistosoma mansoni* the release of immunosuppressive neuropeptides has been associated with deactivation of previously activated host lymphocytes, an example of helminth immunodulation via molecular mimicry, a phenomenon whereby parasites mimic endogenous host proteins in order to interfere with the host response (Duvaux-Miret *et al*. 1992). The greater abundance of the CBR-PGAL-1 homologue transcript in larvae exposed to the immune host environment as opposed to those exposed to the naïve environment raises the possibility that this transcript is involved in neuropeptide processing in response to the host immune factors present, and may play a role in either evasion and/or modulation of the host immune response, or may be involved in changes in developmental regulation related to environmental cues.

The second most abundant transcript in the larvae exposed to the immune abomasal environment (named 'Cluster 611') was exclusive to that dataset and database searches (Wormbase, 2011) indicated significant homology to *C*. *elegans* mitochondrial DNA.

Another transcript present in higher abundance in the immune-exposed larvae, which may be indicative of a response to encountering the immune host environment, was an IDA-1 homologue (in *C*. *remanei*), which is a tyrosine phosphatase-like receptor protein. The *C*. *elegans* homologue of CRE-IDA-1 is a membrane-spanning protein associated with the granules of neural- and endocrine-specific cells, involved in the release of neuropeptides from dense core vesicles, with parallels to insulin signalling in mammals (Zahn *et al*. 2001; Cai *et al*. 2004). An insulin-like pathway is known to regulate dauer formation in *C*. *elegans* following environmental cues (Thomas *et al*. 1993; Gottlieb and Ruvkun, 1994; Riddle and Albert, 1997; Hu, 2007). The *T*. *circumcincta* larvae described herein are entering an environment known to cause immune-mediated inhibition of larval development (McKellar, 1993; Halliday *et al*. 2007; Smith, 2007) therefore the possibility also exists of the IDA-1 homologue protein being involved in a signalling pathway involved in arrested development in response to environmental cues.

Other transcripts with a statistically significantly higher mean abundance in the immune-exposed larvae included the stress-induced heat shock protein 16-2 (HSP 16-2; (Ding and Candido, 2000)). The host immune system will be producing oxidative free radicals to kill pathogens, and HSP 16-2 has been found to protect cells from death due to such oxidative stress (Bellyei *et al*. 2007; Hartwig *et al*. 2009). A *C*. *remanei* ALDO-2 (fructose bisphosphase aldolase) homologue and an *H*. *contortus* phosphoenolpyruvate carboxykinase homologue also displayed greater abundance in the immune-exposed larvae, indicating increased carbon utilization.

Amongst the list of indeterminate Clusters with $P_F \leqslant 0{\cdot}05$ only under the liberal model, sequences showing higher abundance of reads in immune-exposed larvae included a Late Embryo Abundant protein homologue (LEA-1) and *T*. *circumcincta* GTP-Cyclohydrolase. The *lea-1* gene encodes a protein that is involved in the stress-response mechanism in *C*. *elegans*, particularly dessication, osmotic and heat stress (Gal *et al*. 2004). GTP-cyclohydrolase (GTP-CH) has been identified as one of the most abundant transcripts in the exsheathed L3 *T*. *circumcincta* transcriptome when compared to the 4th larval stage (Nisbet *et al*. 2008), and the *C*. *elegans cat-4* gene which encodes GTP-cyclohydrolase shows increased transcription in *C*. *elegans* dauer larvae (Jeong *et al*. 2009). GTP-cyclohydrolase has a proposed role in environmental protection in *T*. *circumcincta* larval stages with transcript levels observed to fall in *T*. *circumcincta* upon transition to the parasitic lifestyle (Baker *et al*. 2011), thus raising the possibility that maintenance of higher levels of transcription in larvae exposed to the immune environment in these experiments may reflect a requirement for ongoing environmental protection.

Within the sequences which had a statistically significantly higher mean proportion of reads in the larvae exposed to the naïve host environment were transcripts for proteins involved in the respiratory electron transport chain; a *C*. *elegans* EGg Laying defective family member (egl-21) homologue (a carboxypeptidase which facilitates acetylcholine release at neuromuscular junctions (Jacob and Kaplan, 2003)); and a *C*. *elegans* C-type LECtin family member (clec-1) homologue with associated biological process Gene Ontologies of body morphogenesis, growth, hermaphrodite genitalia development, locomotion, morphogenesis of an epithelium, nematode larval development and positive regulation of multicellular organism growth. These transcripts which are more abundant in the larvae exposed to the naïve host environment, are consistent with parasites growing and developing as would be predicted given current knowledge of the *T*. *circumcincta* life cycle.

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Table 7. Identities of indeterminate Clusters with statistically significant differences in mean read count abundance between immune-exposed and naïve-exposed groups under the liberal model

(a, Blastx search using representative sequence as query; -, no hits in NCBInr database.)

	Read counts			Top hit protein in NCBI nr database*				
Cluster	Naïve exposed	Immune exposed	*P* (FDR adjusted)	Species	Identity	Accession	*E*-value	Rank (/77)
Clusters containing more reads originating from immune exposed larvae								
Cluster 6	696	957	<0·001	*Caenorhabditis remanei*	CRE-EFT-2 protein	XP_003105057	0	2
Cluster 6854	0	27	<0·001	—	—	—	—	5
Cluster 2363	0	25	<0·001	—	—	—	—	6
Cluster 289	2	91	<0·001	*Brachionus plicatilis*	LEA-like protein 1	ADE05593	3.94E-06	10
Cluster 1929	5	52	<0·001	—	—	—	—	14
Cluster 1805	0	20	<0·001	*Caenorhabditis briggsae*	hypothetical protein	XP_002640908	2·05E-72	15
Cluster 2	1078	1308	<0·001	*Tetrahymena thermophila*	senescence-associated protein	XP_001028745	1.66E-58	17
Cluster 614	292	409	0·001	*Teladorsagia circumcincta*	GTP-Cyclohydrolase	CBX89810.1	5.00E-111	20
Cluster 4966	0	17	0·001	—	—	—	—	21
Cluster 2994	0	16	0·002	*Caenorhabditis remanei*	hypothetical protein CRE_12628	XP_003107924.1	3.85E-38	25
Cluster 616	5	35	0·006	*Caenorhabditis briggsae AF16*	hypothetical protein CBG_22750	CAP39270.2	8.76E-71	32
Cluster 71	3	34	0·007	*Caenorhabditis remanei*	hypothetical protein CRE_02279	XP_003117095.1	1.58E-161	33
Cluster 461	0	14	0·007	*Caenorhabditis elegans*	Guanylyl CYclase family member	NP_497026.2	3.72E-68	34
Cluster 1117	0	14	0·007	—	—	—	—	35
Cluster 1658	0	14	0·007	—	—	—	—	36
Cluster 5579	0	14	0·007	*Angiostrongylus cantonensis*	putative Dynein Heavy Chain	CAR63664.1	3.95E-30	37
Cluster 72	63	115	0·013	*Caenorhabditis briggsae*	CBR-BRP-1 protein	XP_002642206.1	2.37E-16	40
Cluster 4063	0	13	0·013	—	—	—	—	41
Cluster 5627	0	13	0·013	*Caenorhabditis elegans*	hypothetical protein Y57E12AL.1	NP_741562	1.27E-36	42
Cluster 6795	0	13	0·013	—	—	—	—	43
Cluster 1593	1	46	0·016	*Brugia malayi*	hypothetical protein Bm1_25640	XP_001896587.1	1.95E-46	45
Cluster 458	2	31	0·018	*Caenorhabditis remanei*	hypothetical protein CRE_25163	XP_003113223	1.60E-51	46
Cluster 564	0	12	0·023	*Caenorhabditis elegans*	hypothetical protein T12A2.2	NP_498362.1	8.19E-118	48
Cluster 615	0	12	0·023	*Brugia malayi*	UDP-glucoronosyl and UDP-glucosyl	XP_001894161.1	2.03E-59	49
Cluster 878	0	12	0·023	*Caenorhabditis remanei*	hypothetical protein CRE_25063	XP_003113058.1	1.44E-12	50
Cluster 1436	0	12	0·023	*Caenorhabditis briggsae AF16*	hypothetical protein CBG_27997	CAS00988.1	6.71E-48	51
Cluster 2627	0	12	0·023	—	—	—	—	52
Cluster 813	94	150	0·028	*Teladorsagia circumcincta*	NADH dehydrogenase subunit II	YP_003434086.1	3.33E-55	60
Cluster 23	16	45	0·031	*Caenorhabditis elegans*	hypothetical protein C34D4.14	NP_501120.1	0	61
Cluster 146	0	11	0·036	*Caenorhabditis remanei*	hypothetical protein CRE_24093	XP-003099813.1	1.19E-82	63
Cluster 197	0	11	0·036	*Caenorhabditis elegans*	Dynein Heavy Chain family member	NP_491363.1	7.73E-153	64
Cluster 430	0	11	0·036	*Caenorhabditis briggsae*	hypothetical protein CBG21629	XP_002632697.1	5.15E-113	65
Cluster 474	0	11	0·036	*Caenorhabditis elegans*	UNCoordinated family member	NP_001023017.1	8.38E-50	66
Cluster 900	0	11	0·036	*Caenorhabditis elegans*	hypothetical protein F15C11.2	NP_740883.1	6.34E-13	67
Cluster 1040	0	11	0·036	*Caenorhabditis elegans*	hypothetical protein E01G6.3	NP_510042.2	8.75E-25	68
Cluster 1192	0	11	0·036	*Caenorhabditis briggsae AF16*	hypothetical protein CBG_00943	CAP22305.2	5.93E-67	69
Cluster 1827	0	11	0·036	*Caenorhabditis briggsae*	hypothetical protein CBG12633	XP_002640134.1	6.04E-54	70

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Cluster 2983	0	11	0·036	*Steinernema carpocapsae*	saposin precursor	ADG63466.1	7.70E-42	71
Cluster 3175	0	11	0·036	*Loa loa*	hypothetical protein LOAG_04581	EFO23902.1	6.64E-62	72
Cluster 847	4	25	0·047	*Caenorhabditis remanei*	hypothetical protein CRE_15976	XP_003106503.1	1.95E-62	77
Clusters containing more reads originating from naïve exposed larvae								
Cluster 6	696	957	<0·001	*Caenorhabditis remanei*	CRE-EFT-2 protein	XP_003105057	0	2
Cluster 6854	0	27	<0·001	—	—	—	—	5
Cluster 2363	0	25	<0·001	—	—	—	—	6
Cluster 289	2	91	<0·001	*Brachionus plicatilis*	LEA-like protein 1	ADE05593	3.94E-06	10
Cluster 1929	5	52	<0·001	—	—	—	—	14
Cluster 1805	0	20	<0·001	*Caenorhabditis briggsae*	hypothetical protein CBG00466	XP_002640908	2.05E-72	15
Cluster 2	1078	1308	<0·001	*Tetrahymena thermophila*	senescence-associated protein	XP_001028745	1.66E-58	17
Cluster 614	292	409	0·001	*Teladorsagia circumcincta*	GTP-Cyclohydrolase	CBX89810.1	5.00E-111	20
Cluster 4966	0	17	0·001	—	—	—	—	21
Cluster 2994	0	16	0·002	*Caenorhabditis remanei*	hypothetical protein CRE_12628	XP_003107924.1	3.85E-38	25
Cluster 616	5	35	0·006	*Caenorhabditis briggsae AF16*	hypothetical protein CBG_22750	CAP39270.2	8.76E-71	32
Cluster 71	3	34	0·007	*Caenorhabditis remanei*	hypothetical protein CRE_02279	XP_003117095.1	1.58E-161	33
Cluster 461	0	14	0·007	*Caenorhabditis elegans*	Guanylyl CYclase family member	NP_497026.2	3.72E-68	34
Cluster 1117	0	14	0·007	—	—	—	—	35
Cluster 1658	0	14	0·007	—	—	—	—	36
Cluster 5579	0	14	0·007	*Angiostrongylus cantonensis*	putative Dynein Heavy Chain	CAR63664.1	3.95E-30	37
Cluster 72	63	115	0·013	*Caenorhabditis briggsae*	CBR-BRP-1 protein	XP_002642206.1	2.37E-16	40
Cluster 4063	0	13	0·013	—	—	—	—	41
Cluster 5627	0	13	0·013	*Caenorhabditis elegans*	hypothetical protein Y57E12AL.1	NP_741562	1.27E-36	42
Cluster 6795	0	13	0·013	—	—	—	—	43
Cluster 1593	1	46	0·016	*Brugia malayi*	hypothetical protein Bm1_25640	XP_001896587.1	1.95E-46	45
Cluster 458	2	31	0·018	*Caenorhabditis remanei*	hypothetical protein CRE_25163	XP_003113223	1.60E-51	46
Cluster 564	0	12	0·023	*Caenorhabditis elegans*	hypothetical protein T12A2.2	NP_498362.1	8.19E-118	48
Cluster 615	0	12	0·023	*Brugia malayi*	UDP-glucoronosyl and UDP-glucosyl	XP_001894161.1	2.03E-59	49
Cluster 878	0	12	0·023	*Caenorhabditis remanei*	hypothetical protein CRE_25063	XP_003113058.1	1.44E-12	50
Cluster 1436	0	12	0·023	*Caenorhabditis briggsae AF16*	hypothetical protein CBG_27997	CAS00988.1	6.71E-48	51
Cluster 2627	0	12	0·023	—	—	—	—	52
Cluster 813	94	150	0·028	*Teladorsagia circumcincta*	NADH dehydrogenase subunit II	YP_003434086.1	3.33E-55	60
Cluster 23	16	45	0·031	*Caenorhabditis elegans*	hypothetical protein C34D4.14	NP_501120.1	0	61
Cluster 146	0	11	0·036	*Caenorhabditis remanei*	hypothetical protein CRE_24093	XP-003099813.1	1.19E-82	63
Cluster 197	0	11	0·036	*Caenorhabditis elegans*	Dynein Heavy Chain family member	NP_491363.1	7.73E-153	64
Cluster 430	0	11	0·036	*Caenorhabditis briggsae*	hypothetical protein CBG21629	XP_002632697.1	5.15E-113	65
Cluster 474	0	11	0·036	*Caenorhabditis elegans*	UNCoordinated family member	NP_001023017.1	8.38E-50	66
Cluster 900	0	11	0·036	*Caenorhabditis elegans*	hypothetical protein F15C11.2	NP_740883.1	6.34E-13	67
Cluster 1040	0	11	0·036	*Caenorhabditis elegans*	hypothetical protein E01G6.3	NP_510042.2	8.75E-25	68
Cluster 1192	0	11	0·036	*Caenorhabditis briggsae AF16*	hypothetical protein CBG_00943	CAP22305.2	5.93E-67	69
Cluster 1827	0	11	0·036	*Caenorhabditis briggsae*	hypothetical protein CBG12633	XP_002640134.1	6.04E-54	70
Cluster 2983	0	11	0·036	*Steinernema carpocapsae*	saposin precursor	ADG63466.1	7.70E-42	71
Cluster 3175	0	11	0·036	*Loa loa*	hypothetical protein LOAG_04581	EFO23902.1	6.64E-62	72
Cluster 847	4	25	0·047	*Caenorhabditis remanei*	hypothetical protein CRE_15976	XP_003106503.1	1.95E-62	77

* Representative sequence from cluster used as query for Blastx search of NCBI non-redundant database.
— no hits in NCBI nr database.

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Table 8. Proteins exclusively present in either immune or naïve abomasal environment samples, identified by LC-MS/MS

(A match was considered significant when the number of non-redundant peptides with 4 or more consecutive *b* or *y* ions was greater than or equal to 2. a, For each sequence that provided a significant match, only the gel slice containing the highest MOWSE value for that protein is indicated. b, Sequence coverage is expressed as the number of amino acids spanned by the assigned peptide divided by the sequence length. c, The total number of non-redundant peptides assigned to the protein is shown. When calculating this number, multiple matches to peptides with the same primary sequence counted as 1, even if they represented different charge states or modification states. d, The Accession number and identity of the highest hit identified in the NCBI non-redundant database is shown.)

Gel slice[a]	MOWSE score	Sequence coverage (%)[b]	Total no. peptides[c]	GenInfo Identifier[d]	Species	Identity
Immune abomasal environment						
2	152	2	9	gi|40849908	*Mus musculus*	plectin 1
4	286	20	23	gi|114669627	*Pan troglodytes*	PREDICTED: clathrin heavy chain 1 isoform 7
5	396	29	20	gi|297471223	*Bos taurus*	PREDICTED: ceruloplasmin (ferroxidase)
5	60	7	5	gi|73990367	*Canis familiaris*	PREDICTED: similar to Ceruloplasmin precursor (Ferroxidase) isoform 1
5	93	3	3	gi|76677897	*Bos taurus*	complement factor H precursor
6	88	6	3	gi|110331845	*Bos taurus*	inter-alpha (globulin) inhibitor H3
6	66	2	2	gi|149056486	*Rattus norvegicus*	rCG54015
7	147	13	8	gi|114583316	*Pan troglodytes*	PREDICTED: villin 1 isoform 2
7	242	29	16	gi|296190199	*Callithrix jacchus*	PREDICTED: transitional endoplasmic reticulum ATPase-like
7	112	7	6	gi|297462670	*Bos taurus*	PREDICTED: primary amine oxidase, liver isozyme-like
7	107	6	3	gi|114626425	*Pan troglodytes*	PREDICTED: gelsolin isoform 3
7	83	3	2	gi|297290486	*Macaca mulatta*	PREDICTED: complement factor B isoform 1
8	49	11	4	gi|165926	*Ovis aries*	immunoglobulin epsilon-chain
8	66	6	2	gi|191765	*Mus musculus*	alpha-fetoprotein
8	77	5	4	gi|301777396	*Ailuropoda melanoleuca*	PREDICTED: inhibitor of carbonic anhydrase-like
8	226	18	8	gi|149038929	*Rattus norvegicus*	gelsolin, isoform CRA_b
8	241	22	10	gi|296212544	*Callithrix jacchus*	PREDICTED: gelsolin-like isoform 1
8	259	21	10	gi|38044288	*Homo sapiens*	gelsolin isoform b
9	59	5	2	gi|194038353	*Sus scrofa*	PREDICTED: similar to alpha-1-antichymotrypsin 2
9	150	19	8	gi|27806351	*Bos taurus*	ezrin
9	53	8	3	gi|27807349	*Bos taurus*	factor XIIa inhibitor precursor
11	91	18	5	gi|15987978	*Oryctolagus cuniculus*	Chain A, S402p Mutant Of Rabbit Muscle Pyruvate Kinase
11	197	34	10	gi|194670470	*Bos taurus*	PREDICTED: pyruvate kinase, muscle
11	134	18	6	gi|31981562	*Mus musculus*	pyruvate kinase isozymes M1/M2
11	102	8	3	gi|357	*Bos taurus*	fibrinogen beta chain
11	56	10	5	gi|77736171	*Bos taurus*	hemopexin precursor
12	106	6	4	gi|167045824	*Callithrix jacchus*	ATPase, H+ transporting, lysosomal accessory protein 1 precursor (predicted)
12	72	10	2	gi|881556	*Mustela putorius*	fibrinogen, gamma A chain

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12	72	7	3	gi|31092	*Homo sapiens*	unnamed protein product, PROVISIONAL elongation factor 1-alpha
13	128	22	5	gi|213021132	*Ovis aries*	pigment epithelium-derived factor
13	260	40	12	gi|52783777	*Equus caballus*	RecName: Full = Phosphoglycerate kinase 1
13	145	28	5	gi|125975938	*Trichosurus vulpecula*	CP4 protein
13	162	18	6	gi|148709584	*Mus musculus*	mCG50578 dead box helicase
13	176	18	6	gi|4758112	*Homo sapiens*	spliceosome RNA helicase BAT1
15	376	48	12	gi|145308887	*Ovis aries*	intelectin 2
15	293	52	13	gi|157886697	*Ovis aries*	intelectin-3
15	103	9	2	gi|281338720	*Ailuropoda melanoleuca*	hypothetical protein PANDA_018257 Trans-Isoprenyl Diphosphate Synthase region
15	116	26	10	gi|297481614	*Bos taurus*	PREDICTED: prostaglandin F synthase 2-like
15	148	32	11	gi|30794344	*Bos taurus*	dihydrodiol dehydrogenase 3
17	85	12	3	gi|194037097	*Sus scrofa*	carbonic anhydrase 2
17	129	33	5	gi|41406048	*Bos taurus*	chymotrypsin-like elastase family member 1 precursor
17	93	15	3	gi|291410030	*Oryctolagus cuniculus*	PREDICTED: NADPH:secondary-alcohol oxidoreductase-like
19	57	16	2	gi|77735599	*Bos taurus*	ribosyldihydronicotinamide dehydrogenase
19	152	42	5	gi|61553385	*Bos taurus*	heat shock 27 kDa protein 1
19	141	17	5	gi|3329498	*Mus musculus*	heterogenous nuclear ribonucleoprotein A2/B1
20	102	12	2	gi|114051137	*Bos taurus*	tetranectin precursor
20	111	26	4	gi|126305670	*Monodelphis domestica*	PREDICTED: similar to proliferation associated gene (pag)
20	217	52	6	gi|291415601	*Oryctolagus cuniculus*	PREDICTED: cofilin 1 (non-muscle)-like, partial
20	69	5	2	gi|296477855	*Bos taurus*	galectin-4
20	79	–	2	gi|126336522	*Monodelphis domestica*	PREDICTED: similar to RAB43
20	89	10	2	gi|296478535	*Bos taurus*	RAB35, member RAS oncogene family
20	84	20	3	gi|4389379	*Homo sapiens*	Chain A, Cdc42hs-Gdp Complex
21	107	27	4	gi|119901287	*Bos taurus*	PREDICTED: transgelin 2
21	140	14	4	gi|169163387	*Homo sapiens*	PREDICTED: similar to TRIM5/CypA fusion protein
21	183	57	7	gi|281347999	*Ailuropoda melanoleuca*	hypothetical protein PANDA_001384 Arf-1-Arf-5-like subfamily region
21	120	24	2	gi|441486	*Bos taurus*	coatomer, Clathrin adaptor complex small chain region
21	132	37	5	gi|74225050	*Mus musculus*	unnamed protein product, RhoA_like region
22	103	19	3	gi|152207470	*Capra hircus*	galectin 15
22	79	29	3	gi|4507793	*Homo sapiens*	ubiquitin-conjugating enzyme E2 N
24	54	21	2	gi|15216177	*Bos taurus*	putative 42-9-9 protein
24	72	41	4	gi|163315	*Bos taurus*	lysozyme 2a precursor
24	222	60	6	gi|34810222	*Capra hircus*	Chain A, Crystal Structure Of The Mammalian Tumor Associated Antigen Uk114
24	89	22	3	gi|73974071	*Canis lupus familiaris*	PREDICTED: similar to Ribonuclease UK114 (14·5 kDa translational inhibitor protein) isoform 3
26	55	3	2	gi|27806447	*Bos taurus*	proactivator polypeptide (prosaposin)

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Table 8. (*Cont.*)

Gel slice[a]	MOWSE score	Sequence coverage (%)[b]	Total no. peptides[c]	GenInfo Identifier[d]	Species	Identity
Naïve abomasal environment						
7	70	4	2	gi|27806815	*Bos taurus*	plasminogen precursor
7	71	4	2	gi|67846016	*Ovis aries*	glycogen phosphorylase, liver form
7	71	3	2	gi|291395030	*Oryctolagus cuniculus*	PREDICTED: type 1 tumor necrosis factor receptor shedding aminopeptidase regulator
7	56	—	2	gi|3282771	*Homo sapiens*	actin binding protein homologue ABP-278
7	62	7	4	gi|4321127	*Homo sapiens*	IgG Fc binding protein
7	75	18	8	gi|55217	*Mus musculus*	murine valosin-containing protein
8	66	6	3	gi|1170383	*Cricetulus griseus*	RecName: Full = Heat shock protein HSP 90-alpha
8	66	—	3	gi|123681	*Mus musculus*	heat shock protein 90 beta
8	69	7	5	gi|27807263	*Bos taurus*	endoplasmin precursor hsp90 beta
8	143	9	4	gi|194223978	*Equus caballus*	PREDICTED: threonyl-tRNA synthetase
9	69	10	3	gi|31283	*Homo sapiens*	unnamed protein product
10	56	6	2	gi|157074114	*Bos taurus*	plastin-1 a
12	141	22	10	gi|118494	*Equus caballus*	RecName: Full = Retinal dehydrogenase 1; AltName: Aldehyde dehydrogenase
12	66	7	3	gi|194036227	*Sus scrofa*	Selenium-binding protein 1
12	101	10	3	gi|46485440	*Rattus norvegicus*	glucose-6-phosphate isomerase
12	85	23	6	gi|57164383	*Ovis aries*	antithrombin-III precursor
12	69	11	2	gi|1083124	*Ovis aries*	Ig mu heavy chain V region precursor – sheep (fragment)
12	134	9	3	gi|837	*Bos taurus*	unnamed protein product, RNA binding domain
13	178	19	7	gi|114051782	*Bos taurus*	4-trimethylaminobutyraldehyde dehydrogenase
13	162	15	5	gi|435487	*Homo sapiens*	aldehyde dehydrogenase (NAD+)
13	147	38	10	gi|78042564	*Bos taurus*	cytosolic non-specific dipeptidase
13	412	42	15	gi|285975	*Homo sapiens*	human rab GDI
13	176	31	12	gi|4929854	*Homo sapiens*	Chain A, Structure Of Human Placental S-Adenosylhomocysteine Hydrolase
14	224	54	4	gi|163101	*Bos taurus*	glutamine synthetase
15	233	45	11	gi|160286558	*Oryctolagus cuniculus*	Chain A, Fructose 1,6-Bisphosphate Aldolase From Rabbit Muscle
15	198	22	7	gi|229503	*Oryctolagus cuniculus*	aldolase
15	206	37	8	gi|57526769	*Ovis aries*	chymosin precursor
16	231	37	9	gi|147900301	*Bos taurus*	biliverdin reductase A
16	148	27	5	gi|149743872	*Equus caballus*	PREDICTED: similar to 3(2), 5-bisphosphate nucleotidase 1
16	133	34	6	gi|165875541	*Ovis aries*	transaldolase 1
16	137	16	5	gi|194209508	*Equus caballus*	PREDICTED: similar to MGC155110 protein, biliverdin reductase region
16	269	10	3	gi|2947100	*Sus scrofa*	15-oxoprostaglandin 13-reductase
16	341	26	8	gi|296484379	*Bos taurus*	prostaglandin reductase 1
16	150	–	3	gi|56789674	*Mus musculus*	farnesyl diphosphate synthetase
16	197	31	6	gi|77736025	*Bos taurus*	3′(2′),5′-bisphosphate nucleotidase 1

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17	233	27	8	gi|115495641	*Bos taurus*	alcohol dehydrogenase [NADP+]
17	252	34	6	gi|115497074	*Bos taurus*	S-formylglutathione hydrolase
17	342	36	10	gi|300796734	*Bos taurus*	annexin A10
17	96	10	2	gi|301764565	*Ailuropoda melano*	PREDICTED: LOW QUALITY PROTEIN: isochorismatase domain-containing protein 1-like
17	108	33	4	gi|4929691	*Homo sapiens*	CGI-111 protein
17	208	22	6	gi|6754524	*Mus musculus*	L-lactate dehydrogenase A chain isoform 1
17	89	8	2	gi|74002351	*Canis familiaris*	PREDICTED: similar to caspase 6 isoform alpha preproprotein
17	378	35	9	gi|77736203	*Bos taurus*	malate dehydrogenase, cytoplasmic
17	215	19	5	gi|78042488	*Bos taurus*	adenylyl cyclase-associated protein 1
17	103	20	4	gi|126337842	*Monodelphis domestica*	PREDICTED: similar to p34-Arc
19	84	14	2	gi|4504771	*Homo sapiens*	eukaryotic translation initiation factor 6 isoform a
19	81	27	4	gi|7106387	*Mus musculus*	proteasome subunit alpha type-5
20	149	41	5	gi|240849225	*Ovis aries*	adenylate kinase isoenzyme 1
20	111	25	4	gi|2497487	*Sus scrofa*	RecName: Full = UMP-CMP kinase; AltName: Full = Cytidine monophosphate kinase
20	186	39	4	gi|28189813	*Bos taurus*	similar to translationally controlled tumor protein
20	113	20	3	gi|32401410	*Bos taurus*	immunoglobulin J chain
20	83	34	2	gi|4322114	*Isoodon macrourus*	hypoxanthine phosphoribosyltransferase HPRT-1
20	85	–	2	gi|77404180	*Rattus norvegicus*	Ras-related small GTP binding protein 4
21	111	47	6	gi|296488630	*Bos taurus*	anterior gradient 2 homolog
21	97	11	2	gi|16758368	*Rattus norvegicus*	ras-related protein Rab-14
22	118	22	2	gi|4758442	*Homo sapiens*	glia maturation factor beta
23	150	42	6	gi|114051526	*Bos taurus*	coactosin-like protein
23	103	37	3	gi|1293786	*Bos taurus*	LP2, differentiation-associated lipid binding protein
24	501	79	8	gi|253723066	*Bos taurus*	Chain A, Crystallization And Structure Determination Of Bovine Profilin
25	90	61	5	gi|5031985	*Homo sapiens*	nuclear transport factor 2
25	80	25	2	gi|1942335	*Bos taurus*	Chain C, Ternary Complex Of A Calcineurin A Fragment, Calcineurin B, Fkbp12 And The Immunosuppressant Drug Fk506 (Tacrolimus)
25	60	25	2	gi|197724894	*Homo sapiens*	Chain A, Crystal Structure Of E60q Mutant Of Fkbp12 – FKBP-type peptidyl-prolyl cis-trans isomerase
26	69	26	3	gi|545707	*Bos taurus*	Capl = S-100-related calcium-binding protein

The most abundant transcript in each larval dataset was a *B. malayi* hypothetical protein homologue with similarity to a checkpoint-like protein from *Helicoverpa armigera* (cotton bollworm) which shows increased expression during larval molting (Dong *et al*. 2007). Here, the larvae in both treatments were exsheathing during the incubation period and the presence of high levels of this transcript may reflect this process.

Transcriptomic analysis of the parasitic nematode *Strongyloides ratti* (O'Meara *et al*. 2010; Thompson *et al*. 2008) showed an increase in expression of an IDA family member (*ida*-1) and a small heat shock protein family member (*C. elegans* HSP 17) in response to host immunity and high immune pressure respectively, results comparable to those presented here. Interestingly, in *S. ratti unc*-54 and a C-type lectin family member (*clec*-200) were also upregulated in response to host immunity, whereas these transcripts were more abundant in *T. circumcincta* larvae exposed to a naïve host environment. The *S. ratti* transcriptomic study was carried out 12 days after infection as opposed to after 4 h exposure to a naïve or immune environment, which may explain the differences observed.

KEGG biological pathways with a statistically significantly increased mean proportion of Isotigs in the immune-exposed larval sample included carbohydrate and energy metabolism, and biosynthesis of alkaloids and secondary metabolites. An increase in energy metabolism could represent an increased requirement for energy to mount responses to the host environment. The biosynthesis of alkaloids, phenylpropanoids and other secondary metabolites is known to be involved in response to environmental stress in plants (Iriti and Faoro, 2009), and herein could be representative of a stress response in the immune environment-exposed larvae accompanied by contemporaneous adjustment in metabolic pathways.

The method employed here to create the abomasal environments *in vitro* will not result in an exact replica of the physiological situation that the larvae encounter *in vivo*. Analysis of the protein profiles of these abomasal samples showed differences between the environments created using the abomasa of naïve and immune sheep, these being comparable to the results obtained from in-depth quantitative analysis of the equivalent abomasal tissue (Athanasiadou *et al*. 2008; Alan Pemberton, *personal communication*). One of the proteins with the highest number of non-redundant peptides identified from immune sheep was clathrin, a prominent protein component of cellular protein transport vesicles with the formation of a clathrin coat being fundamental to the mechanism of vesicle budding as well as receptor sorting and transport specificity (McDowall, 2007). Proteins which are known to be upregulated in the abomasa of immune sheep and which are thought to play a role *in vivo* in local immunity, such as intelectin-2, galectin-15, IgE and gelsolin-b, were all detected exclusively in the immune abomasal environment sample. Sheep intelectin-2 (Itln-2) expression is upregulated in the abomasa of immune sheep following challenge compared to naïve sheep undergoing a primary infection and immunolocalizes to abomasal mucus neck cells and gastric mucus surrounding developing larvae (French *et al*. 2008). Itln-2 co-purifies with the mucin Muc5a from gastric mucosa to alter the rheological properties of the mucus, which could potentially reduce larval penetration to the underlying mucus layer (Pemberton *et al*. 2011). Galectin-15 (ovgal11) shows greatly increased expression in the abomasal tissue of sheep infected with *H. contortus* compared to uninfected tissue, and the mRNA appears more rapidly in previously sensitized sheep compared to naïve sheep (Dunphy *et al*. 2000). Galectin-15 protein is also secreted into the lumen and detected in mucus where it may interact with carbohydrate motifs such as mucins. Increased levels of locally produced IgE have been observed in previously infected sheep undergoing challenge infection when compared to naïve sheep undergoing a primary infection (Huntley *et al*. 1998), and gelsolin levels in ovine gastric lymph have been demonstrated to decrease *in vivo* in naïve sheep undergoing a primary infection but not during challenge of immune sheep (Goldfinch *et al*. 2008). Gelsolin can function as an actin scavenger, possibly binding free actin released from damaged cells to prevent actin-polymerization and subsequent detrimental effects on mucus viscosity (Sun *et al*. 1999; Goldfinch *et al*. 2008). It is also known to be downregulated as cells become less differentiated, for example during replacement of acid-producing parietal cells with non-differentiated non-acid secreting cells as is seen in the abomasum during *T. circumcincta* infection, and may also have a role in the regulation of mucus secretion from goblet cells (Vandekerckhove *et al*. 1990; Ehre *et al*. 2005). Detection of these proteins exclusively in the immune abomasal environment sample suggests that the abomasal environment fluids used for larval culture here were at least partly representative of the soluble abomasal proteome.

An astacin-like metalloprotease was identified by Smith *et al*. (2009) in the excretory/secretory (ES) products of *T. circumcincta* larvae recovered 1 day post-infection of naïve sheep; however, transcript for this protein was not detected in either of the larval datasets generated here. It is possible that production of this protein had not yet commenced within the 4 h of exposure to the abomasal environment as compared to the 24+ h exposure described by Smith *et al*. (2009).

In conclusion, this paper defines the major transcriptional changes in *T. circumcincta* upon encountering either a naïve or an immune ovine

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host environment. Statistically significant differences in mean transcript levels between the 2 larval populations were detected for several genes with possible roles in sensory capabilities and response to the differing environments. The larvae exposed to the immune environment are encountering conditions known to lead to larval inhibition, therefore the possibility exists that the transcriptional changes observed may relate to larvae entering a state of arrest. Further work could focus on localization and functional analysis of these proteins to further determine the response of the parasites to varying host environments and concomitant effects on parasitic behaviour and development.

DATA

454 sequencing data are available in the Sequencing Read Archive (SRA), Accession number ERP000810, http://www.ebi.ac.uk/ena/data/view/ERP000810.

Assembled transcript data are available via the PartiGene database http://genepool.bio.ed.ac.uk/GP_Partigene/2010038_AileenHalliday_AH15AH16/ (Parkinson *et al*. 2004).

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REFERENCES

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M. and Sherlock, G. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics* **25**, 25–29.

Athanasiadou, S., Pemberton, A., Jackson, F., Inglis, N., Miller, H., Thevenod, F., Mackellar, A. and Huntley, J. (2008). Proteomic approach to identify candidate effector molecules during the in vitro immune exclusion of infective *Teladorsagia circumcincta* in the abomasum of sheep. *Veterinary Research* **39**, 58–62.

Baker, R. H., Buschbaum, S., Matthews, J. B., McKendrick, I. J., Schnieder, T., Strube, C. and Nisbet, A. J. (2011). GTP-Cyclohydrolase and development in *Teladorsagia circumcincta* and *Dictyocaulus viviparus* (Nematoda: Strongylida). *Experimental Parasitology* **128**, 309–317.

Balic, A., Bowles, V. M., Liu, Y. S. and Meeusen, E. N. T. (2003). Local immune responses in sensitized sheep following challenge infection with *Teladorsagia circumcincta*. *Parasite Immunology* **25**, 375–381.

Bellyei, S., Szigeti, A., Boronkai, A., Pozsgai, E., Gomori, E., Melegh, B., Janaky, T., Bognar, Z., Hocsak, E., Sumegi, B. and Gallyas, F. Jr. (2007). Inhibition of cell death by a novel 16·2 kD heat shock protein predominantly via Hsp90 mediated lipid rafts stabilization and Akt activation pathway. *Apoptosis* **12**, 97–112.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate – a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological* **57**, 289–300.

Benjamini, Y. and Yekutieli, D. (2005). Quantitative trait loci analysis using the false discovery rate. *Genetics* **171**, 783–790.

Cai, T., Fukushige, T., Notkins, A. L. and Krause, M. (2004). Insulinoma-Associated Protein IA-2, a vesicle transmembrane protein, genetically interacts with UNC-31/CAPS and affects neurosecretion in *Caenorhabditis elegans*. *The Journal of Neuroscience* **24**, 3115–3124.

Cantacessi, C., Campbell, B. E., Young, N. D., Jex, A. R., Hall, R. S., Presidente, P. J., Zawadzki, J. L., Zhong, W., Aleman, B., Loukas, A., Sternberg, P. W. and Gasser, R. B. (2010*a*). Differences in transcription between free-living and CO2-activated third-stage larvae of *Haemonchus contortus*. *BioMed Central Genomics* **11**, 266–279.

Cantacessi, Cinzia, Mitreva, Makedonka, Campbell, Bronwyn E., Hall, Ross S., Young, Neil D., Jex, Aaron R., Ranganathan, Shoba and Gasser, Robin B. (2010*b*). First transcriptomic analysis of the economically important parasitic nematode, *Trichostrongylus colubriformis*, using a next-generation sequencing approach. *Infection, Genetics and Evolution* **10**, 1199–1207.

Conesa, A. and Gotz, S. (2008). Blast2GO: A comprehensive suite for functional analysis in plant genomics. *International Journal of Plant Genomics* **2008**, 1–12.

Conesa, A., Gotz, S., Garcia-Gomez, J. M., Terol, J., Talon, M. and Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21**, 3674–3676.

Dicker, Alison, J., Nath, Mintu, Yaga, Raja, Nisbet, Alasdair, J., Lainson, F., Alex, Gilleard, John, S. and Skuce, Philip, J. (2011). *Teladorsagia circumcincta*: The transcriptomic response of a multi-drug-resistant isolate to ivermectin exposure in vitro. *Experimental Parasitology* **127**, 351–356.

Ding, L. and Candido, E. P. (2000). Association of several small heat-shock proteins with reproductive tissues in the nematode *Caenorhabditis elegans*. *The Biochemical Journal* **351**, 13–17.

Dong, D. J., He, H. J., Chai, L. Q., Jiang, X. J., Wang, J. X. and Zhao, X. F. (2007). Identification of genes differentially expressed during larval molting and metamorphosis of *Helicoverpa armigera*. *BioMed Central Developmental Biology* **7**, 73–88.

Dunphy, J. L., Balic, A., Barcham, G. J., Horvath, A. J., Nash, A. D. and Meeusen, E. N. (2000). Isolation and characterization of a novel inducible mammalian galectin. *The Journal of Biological Chemistry* **275**, 32106–32113.

Duvaux-Miret, O., Stefano, G. B., Smith, E. M., Dissous, C. and Capron, A. (1992). Immunosuppression in the definitive and intermediate hosts of the human parasite *Schistosoma mansoni* by release of immunoactive neuropeptides. *Proceedings of the National Academy of Sciences, USA* **89**, 778–781.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461.

Ehre, C., Rossi, A. H., Abdullah, L. H., De, Pestel, K., Hill, S., Olsen, J. C. and Davis, C. W. (2005). Barrier role of actin filaments in regulated mucin secretion from airway goblet cells. *American Journal for Physiology – Cell Physiology* **288**, C46–C56.

Eipper, B. A., Milgram, S. L., Husten, E. J., Yun, H. Y. and Mains, R. E. (1993). Peptidylglycine alpha-amidating monooxygenase: a multifunctional protein with catalytic, processing, and routing domains. *Protein Science* **2**, 489–497.

French, A. T., Knight, P. A., Smith, W. D., Brown, J. K., Craig, N. M., Pate, J. A., Miller, H. R. P. and Pemberton, A. D. (2008). Up-regulation of intelectin in sheep after infection with *Teladorsagia circumcincta*. *International Journal for Parasitology* **38**, 467–475.

Gal, T. Z., Glazer, I., and Koltai, H. (2004). An LEA group 3 family member is involved in survival of *C. elegans* during exposure to stress. *FEBS Letters* **577**, 21–26.

Goldfinch, G. M., Smith, W. D., Imrie, L., McLean, K., Inglis, N. F. and Pemberton, A. D. (2008). The proteome of gastric lymph in normal and nematode infected sheep. *Proteomics* **8**, 1909–1918.

Gottlieb, S. and Ruvkun, G. (1994). daf-2, daf-16 and daf-23: genetically interacting genes controlling Dauer formation in *Caenorhabditis elegans*. *Genetics* **137**, 107–120.

Gotz, S., Garcia-Gomez, J. M., Terol, J., Williams, T. D., Nagaraj, S. H., Nueda, M. J., Robles, M., Talon, M., Dopazo, J. and

https://doi.org/10.1017/S0031182011002010 Published online by Cambridge University Press

Conesa, A. (2008). High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Research* **36**, 3420–3435.

Halliday, A. M., McAllister, H. C. and Smith, W. D. (2010). Kinetics of the local immune response in the gastric lymph of lambs after primary and challenge infection with *Teladorsagia circumcincta*. *Parasite Immunology* **32**, 81–90.

Halliday, A. M., Morrison, W. I. and Smith, W. D. (2009). Kinetics of the local cellular response in the gastric lymph of immune and susceptible sheep to infection with *Teladorsagia circumcincta*. *Parasite Immunology* **31**, 402–411.

Halliday, A. M., Routledge, C. M., Smith, S. K., Matthews, J. B. and Smith, W. D. (2007). Parasite loss and inhibited development of *Teladorsagia circumcincta* in relation to the kinetics of the local IgA response in sheep. *Parasite Immunology* **29**, 425–434.

Hartwig, K., Heidler, T., Moch, J., Daniel, H. and Wenzel, U. (2009). Feeding a ROS-generator to Caenorhabditis elegans leads to increased expression of small heat shock protein HSP-16.2 and hormesis. *Genes and Nutrition* **4**, 59–67.

Hu, P. J. (2007). Dauer. In *WormBook* (ed. The *C. elegans* Research Community), pp. 1–19. WormBook http://www.wormbook.org

Hunter, S., Apweiler, R., Attwood, T. K., Bairoch, A., Bateman, A., Binns, D., Bork, P., Das, U., Daugherty, L., Duquenne, L., Finn, R. D., Gough, J., Haft, D., Hulo, N., Kahn, D., Kelly, E., Laugraud, A., Letunic, I., Lonsdale, D., Lopez, R., Madera, M., Maslen, J., McAnulla, C., McDowall, J., Mistry, J., Mitchell, A., Mulder, N., Natale, D., Orengo, C., Quinn, A. F., Selengut, J. D., Sigrist, C. J., Thimma, M., Thomas, P. D., Valentin, F., Wilson, D., Wu, C. H. and Yeats, C. (2009). InterPro: the integrative protein signature database. *Nucleic Acids Research* **37**, D211–D215.

Huntley, J. F., Schallig, H. D. F. H., Kooyman, F. N. J., Mackellar, A., Jackson, F. and Smith, W. D. (1998). IgE antibody during infection with the ovine abomasal nematode, *Teladorsagia circumcincta*: primary and secondary responses in serum and gastric lymph of sheep. *Parasite Immunology* **20**, 565–571.

Iriti, M. and Faoro, F. (2009). Chemical diversity and defence metabolism: how plants cope with pathogens and ozone pollution. *International Journal of Molecular Sciences* **10**, 3371–3399.

Jackson, F., Greer, A. W., Huntley, J., McAnulty, R. W., Bartley, D. J., Stanley, A., Stenhouse, L., Stankiewicz, M. and Sykes, A. R. (2004). Studies using *Teladorsagia circumcincta* in an in vitro direct challenge method using abomasal tissue explants. *Veterinary Parasitology* **124**, 73–89.

Jacob, T. C. and Kaplan, J. M. (2003). The EGL-21 carboxypeptidase E facilitates acetylcholine release at *Caenorhabditis elegans* neuromuscular junctions. *The Journal of Neuroscience* **23**, 2122–2130.

Jeong, P. Y., Kwon, M. S., Joo, H. J. and Paik, Y. K. (2009). Molecular time-course and the metabolic basis of entry into dauer in *Caenorhabditis elegans*. *Public Library of Science ONE* **4**, e4162.

Kanehisa, M. and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* **28**, 27–30.

Macaldowie, C., Jackson, F., Huntley, J., Mackellar, A. and Jackson, E. (2003). A comparison of larval development and mucosal mast cell responses in worm-naive goat yearlings, kids and lambs undergoing primary and secondary challenge with *Teladorsagia circumcincta*. *Veterinary Parasitology* **114**, 1–13.

Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., Berka, J., Braverman, M. S., Chen, Y. J., Chen, Z., Dewell, S. B., Du, L., Fierro, J. M., Gomes, X. V., Godwin, B. C., He, W., Helgesen, S., Ho, C. H., Irzyk, G. P., Jando, S. C., Alenquer, M. L., Jarvie, T. P., Jirage, K. B., Kim, J. B., Knight, J. R., Lanza, J. R., Leamon, J. H., Lefkowitz, S. M., Lei, M., Li, J., Lohman, K. L., Lu, H., Makhijani, V. B., McDade, K. E., McKenna, M. P., Myers, E. W., Nickerson, E., Nobile, J. R., Plant, R., Puc, B. P., Ronan, M. T., Roth, G. T., Sarkis, G. J., Simons, J. F., Simpson, J. W., Srinivasan, M., Tartaro, K. R., Tomasz, A., Vogt, K. A., Volkmer, G. A., Wang, S. H., Wang, Y., Weiner, M. P., Yu, P., Begley, R. F. and Rothberg, J. M. (2005). Genome sequencing in microfabricated high-density picolitre reactors. *Nature, Lomdon* **437**, 376–380.

McDowall, J. (2007). Clathrin. http://www.ebi.ac.uk/interpro/potm/2007_4/Page1.htm

McKellar, Q. A. (1993). Interactions of *Ostertagia* species with their bovine and ovine hosts. *International Journal for Parasitology* **23**, 451–462.

Miller, H. R. P. (1996). Mucosal mast cells and the allergic response against nematode parasites. *Veterinary Immunology and Immunopathology* **54**, 331–336.

Moredun (2011). Moredun Research Institute Proteomics Facility. http://www.moredun.org.uk/research/expertise-@-moredun/proteomics.

Nelson, L. S., Rosoff, M. L. and Li, C. (1998). Disruption of a neuropeptide gene, flp-1, causes multiple behavioral defects in *Caenorhabditis elegans*. *Science* **281**, 1686–1690.

Nieuhof, G. J. and Bishop, S. C. (2005). Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. *Animal Science* **81**, 23–29.

Nisbet, A. J., Redmond, D. L., Matthews, J. B., Watkins, C., Yaga, R., Jones, J. T., Nath, M. and Knox, D. P. (2008). Stage-specific gene expression in *Teladorsagia circumcincta* (*Nematoda: Strongylida*) infective larvae and early parasitic stages. *International Journal for Parasitology* **38**, 829–838.

O'Meara, H., Barber, R., Mello, L. V., Sangaralingam, A., Viney, M. E. and Paterson, S. (2010). Response of the *Strongyloides ratti* transcriptome to host immunological environment. *International Journal for Parasitology* **40**, 1609–1617.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H. and Kanehisa, M. (1999). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research* **27**, 29–34.

Parkinson, J., Anthony, A., Wasmuth, J., Schmid, R., Hedley, A. and Blaxter, M. (2004). PartiGene – Constructing partial genomes. *Bioinformatics* **20**, 1398–1404.

Pemberton, A. D., Verdon, B., Inglis, N. F. and Pearson, J. P. (2011). Sheep intelectin-2 co-purifies with the mucin Muc5ac from gastric mucus. *Research in Veterinary Science*. doi: 10.1016/j.rvsc.2011.03.004.

Prigge, S. T., Mains, R. E., Eipper, B. A. and Amzel, L. M. (2000). New insights into copper monooxygenases and peptide amidation: structure, mechanism and function. *Cellular and Molecular Life Sciences* **57**, 1236–1259.

R Development Core Team (2010). A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Release 2.13. http://www.R-project.org.

Riddle, D. L. and Albert, P. S. (1997). *Genetic and Environmental Regulation of Dauer Larva Development*. In *C. elegans II* 2nd Edn. Chapter 26. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Roche (2010). Part C – GS De Novo Assembler, GSS Reference Mapper, SFF Tools. In *454 Sequencing System Software Manual, v 2.5.3* 454 Life Sciences Corp, A Roche Company. Branford CT 06405.

Roche (2011). Roche 454 Website. http://www.454.com.

Sargison, N. D. (2011). Pharmaceutical control of endoparasitic helminth infections in sheep. *Veterinary Clinics of North America: Food Animal Practice* **27**, 139–156.

Scott, P. R., Sargison, N. D. and Wilson, D. J. (2007). The potential for improving welfare standards and productivity in United Kingdom sheep flocks using veterinary flock health plans. *The Veterinary Journal* **173**, 522–531.

Smith, S. K., Nisbet, A. J., Meikle, L. I., Inglis, N. F., Sales, J., Beynon, R. J. and Matthews, J. B. (2009). Proteomic analysis of excretory/secretory products released by *Teladorsagia circumcincta* larvae early post-infection. *Parasite Immunology* **31**, 10–19.

Smith, W. D. (2007). Some observations on immunologically mediated inhibited *Teladorsagia circumcincta* and their subsequent resumption of development in sheep. *Veterinary Parasitology* **147**, 103–109.

Smith, W. D., Jackson, F., Jackson, E., Graham, R., Williams, J., Willadsen, S. M. and Fehilly, C. B. (1986). Transfer of immunity to *Ostertagia circumcincta* and IgA memory between identical sheep by lymphocytes collected from gastric lymph. *Research in Veterinary Science* **41**, 300–306.

Smith, W. D., Jackson, F., Jackson, E. and Williams, J. (1983). Local immunity and *Ostertagia circumcincta*: changes in the gastric lymph of immune sheep after a challenge infection. *Journal of Comparative Pathology* **93**, 479–488.

Stear, M. J., Bishop, S. C., Doligalska, M., Duncan, J. L., Holmes, P. H., Irvine, J., McCririe, L., McKellar, Q. A., Sinski, E. and Murray, M. (1995). Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology* **17**, 643–652.

Stear, M. J., Strain, S. and Bishop, S. C. (1999*a*). How lambs control infection with *Ostertagia circumcincta*. *Veterinary Immunology and Immunopathology* **72**, 213–218.

Stear, M. J., Strain, S. and Bishop, S. C. (1999*b*). Mechanisms underlying resistance to nematode infection. *International Journal for Parasitology* **29**, 51–56.

Sun, H. Q., Yamamoto, M., Mejillano, M. and Yin, H. L. (1999). Gelsolin, a multifunctional actin regulatory protein. *Journal of Biological Chemistry* **274**, 33179–33182.

https://doi.org/10.1017/S0031182011002010 Published online by Cambridge University Press

Thomas, J. H., Birnby, D. A. and Vowels, J. J. (1993). Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. *Genetics* **134**, 1105–1117.

Thompson, F. J., Barker, G. L., Hughes, L. and Viney, M. E. (2008). Genes important in the parasitic life of the nematode *Strongyloides ratti*. *Molecular and Biochemical Parasitology* **158**, 112–119.

Vandekerckhove, J., Bauw, G., Vancompernolle, K., Honore, B. and Celis, J. (1990). Comparative two-dimensional gel analysis and micro-sequencing identifies gelsolin as one of the most prominent downregulated markers of transformed human fibroblast and epithelial cells. *Journal of Cell Biology* **111**, 95–102.

Wormbase (2011). Wormbase website. http://www.wormbase.org. Release WS225.

Young, N. D., Hall, R. S., Jex, A. R., Cantacessi, C. and Gasser, R. B. (2003). Elucidating the transcriptome of *Fasciola hepatica* – A key to fundamental and biotechnological discoveries for a neglected parasite. *Biotechnology Advances* **28**, 222–231.

Zahn, T. R., Macmorris, M. A., Dong, W., Day, R. and Hutton, J. C. (2001). IDA-1, a *Caenorhabditis elegans* homolog of the diabetic autoantigens IA-2 and phogrin, is expressed in peptidergic neurons in the worm. *The Journal of Comparative Neurology* **429**, 127–143.

https://doi.org/10.1017/S0031182011002010 Published online by Cambridge University Press