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Proteomic identification of galectin-11 and -14 ligands from *Fasciola hepatica*

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ABSTRACT

Fasciola hepatica is a globally distributed zoonotic trematode that causes fasciolosis in livestock, wildlife, ruminants and humans. Fasciolosis causes a significant economic impact on the agricultural sector and affects human health. Due to the increasing prevalence of triclabendazole resistance in *F. hepatica*, alternative treatment methods are required. Many protein antigens have been trialled as vaccine candidates with low success, however, the tegument of *F. hepatica* is highly glycosylated and the parasite-derived glycoconjugate molecules have been identified as an important mediator in host-parasite interactions and as prime targets for the host immune system. Galectin-11 (LGALS-11) and galectin-14 (LGALS-14) are two ruminant-specific glycan-binding proteins, showing upregulation in the bile duct of sheep infected with *F. hepatica*, which are believed to mediate host-parasite interaction and innate immunity against internal parasites. For the first known time, this study presents the ligand profile of whole worm and tegument extracts of *F. hepatica* that interacted with immobilised LGALS-11 and LGALS-14. LGALS-14 interacted with a total of 255 *F. hepatica* proteins. The protein which had the greatest interaction was identified as an uncharacterised protein which contained a C-type lectin domain. Many of the other proteins identified were previously trialled vaccine candidates including glutathione S-transferase, paramyosin, cathepsin L, cathepsin B, fatty acid binding protein and leucine aminopeptidase. In comparison to LGALS-14, LGALS-11 interacted with only 49 *F. hepatica* proteins and it appears to have a much smaller number of binding partners in *F. hepatica*. This is, to our knowledge, the first time host-specific lectins have been used for the enrichment of *F. hepatica* glycoproteins and this study has identified a number of glycoproteins that play critical roles in host-parasite interactions which have the potential to be novel vaccine candidates.

1. Introduction

Fasciola hepatica, commonly referred to as "liver fluke", is a globally distributed zoonotic trematode that causes significant economic losses. Although *F. hepatica* infects humans and wildlife, it has the greatest impact on the agricultural sector, causing substantial global production losses in livestock animals of approximately US $3 billion each year (Spithill et al., 1999). Currently, the only anthelmintic treatment approved for use in humans and the most heavily relied upon anthelmintic for liver fluke control in ruminants is triclabendazole (TCBZ); however, TCBZ resistance is now widely reported around the world, with prevalence predicted to increase, making liver fluke an important parasitic infection (Kelley et al., 2016). Developing an effective vaccine for the treatment of *F. hepatica* is a promising and sustainable solution to overcome drug resistance. Many recombinant and native protein vaccines have been trialled but have had variable efficacy (Toet et al., 2014). These have mainly been tegument or excretory/secretory (ES) proteins, as they perform key functions including nutrient absorption and immune evasion. A new approach is directed by the consideration that many of the tegument proteins of *F. hepatica* are highly glycosylated, creating a glycocalyx which could be shielding the underlying proteins from the immune system (Garcia-Campos et al., 2016; Ravidà et al., 2016a,b). Alternatively, hidden glycopro-

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tein antigens could possibly provide a protective response. A family of host molecules which interact with parasite glycoproteins are galectins, which are carbohydrate-binding proteins known to play a role in innate and adaptive immunity (Rabinovich and Toscano, 2009). Two such galectins which are specific to ruminants are galectin 11 (LGALS-11) and galectin 14 (LGALS-14) (Dunphy et al., 2000, 2002).

LGALS-11 is expressed within the nucleus and cytoplasm of epithelial cells, and secreted into the mucus of the gastrointestinal lining (Dunphy et al., 2000). LGALS-11 is a prototypical galectin containing a single carbohydrate recognition domain (Dunphy et al., 2000) and two proposed integrin binding sites (Sakthivel et al., 2015). The specific function of LGALS-11 is currently unknown; however, it has been shown to be upregulated following parasitic infection and is thought to play a role in innate and adaptive immune responses (Hoorens et al., 2011; Robinson et al., 2011) as well as reproduction (Gray et al., 2004; Lewis et al., 2007; Farmer et al., 2008). Due to the secretion of LGALS-11 into mucus linings, its interaction with the gastrointestinal parasite *Haemonchus contortus* has been studied in some detail. LGALS-11 has been shown to bind to the surface of *H. contortus* in a stage-specific manner, only binding to L4 and adult life stages, and to interfere with the exsheathment of the L3 cuticle and L4 growth in vitro, likely due to the LGALS-11 interactions occurring in the pharynx and rectal regions of the worm (Preston et al., 2015a). To date, the specific glycan ligands of LGALS-11 are unknown, however recombinant LGALS-11 has been shown to elute off a galactose-Sepharose column in the presence of galactose, lactose, mannose and fructose, indicating it possibly has a broad glycan specificity (Sakthivel et al., 2015). Recently, Sakthivel et al. (2018) identified a number glycoproteins of adult *H. contortus* which interact with LGALS-11, including zinc metallopeptidase which is a component of the H-gal-GP complex (*Haemonchus* galactose-containing glycoproteins) native vaccine. Other glycoproteins identified include S28 proteases, which share homology with contortin, another previously trialled vaccine candidate (Sakthivel et al., 2018).

LGALS-14 is another ruminant-specific prototype galectin thought to have an immunological role (Dunphy et al., 2002). LGALS-14 is constitutively expressed in the cytoplasm and nucleus of eosinophils and basophils, however, it is only secreted following migration to damaged tissues caused by allergens or parasitic infection (Young et al., 2009). LGALS-14 is known to form homodimers potentially aiding in the formation of lattice structures (Rapoport et al., 2008). Recombinant LGALS-14 is known to bind to glycans containing a low number of N-acetyllactosamine (Galβ1-4GlcNAc) repeats which can be modified by α1-2 fucosylation or α2-sialylation (Young et al., 2009). This includes lacto-N-neotetraose (LNnT), which is a glycan expressed by schistosomes that has been demonstrated to skew the immune response towards a type 2 profile (Terrazas et al., 2001). LGALS-14 has also been shown to interact with 37 *H. contortus* proteins including previously trialled vaccine candidates and immune modulatory molecules such as specific sperm coating protein and von-Willebrand factor domain-containing proteins (Sakthivel et al., 2018).

Although the functions of LGALS-11 and LGALS-14 are more thoroughly understood within the gastrointestinal tract following nematode infection, Young et al. (2012) have shown the presence of LGALS-11 in the epithelial layer and LGALS-14 present in the subepithelial connective tissue of the bile ducts of *F. hepatica*-infected sheep. Additionally, secreted LGALS-11 and LGALS-14 were detected in the bile fluid of sheep infected with *F. hepatica*, with neither galectin detected in uninfected animals (Young et al., 2012). Both galectins are believed to be upregulated following parasitic infection (Hoorens et al., 2011; Robinson et al., 2011; Preston et al., 2015b; Chitneedi et al., 2018). More specifically, LGALS-14 has been observed to be upregulated at two and 8 weeks p.i. in peripheral blood mononuclear cells (PBMCs) of sheep infected with *F. hepatica* (Alvarez Rojas et al., 2016) and from *F. hepatica* damaged liver tissue collected from sheep 8 weeks p.i. (Alvarez Rojas et al., 2015). However, in an allergic airway sheep model, LGALS-14 was shown to be released as soon as 24–48 h post stimulation (Dunphy et al., 2002).

It appears that LGALS-11 and LGALS-14 are upregulated in the host upon infection with *F. hepatica*, however, there is very little known about galectin-parasite glycoconjugate interactions and what effects these mediate. For the first known time, this study uses host-specific lectins (LGALS-11 and LGALS-14) to identify *F. hepatica* glycoproteins in an endeavour to better understand host-parasite interactions.

2. Materials and methods

2.1. Glycoprotein preparation

Adult flukes were collected from naturally infected dairy cattle located in the Macalister Irrigation District (MID), Maffra, Victoria, south-eastern Australia (Kelley et al., unpublished data). Once removed from the bile ducts, flukes were washed three times in PBS. To remove host-derived lectins, flukes were incubated for 2 h at 4 °C in 50 mM galactose and 50 mM lactose. Excess galactose and lactose were removed by rinsing flukes twice in PBS. Tegument extract (FhTeg) was obtained by soaking three flukes, weighing a total of 1 g, in 1.5 ml of 1% sodium deoxycholate for 60 min at 37 °C (Rodríguez et al., 2017). These extracts were prepared in triplicate. To extract the remaining fluke proteins (FhW), flukes (with tegument removed) were snap frozen in liquid nitrogen and homogenised in a pre-cooled mortar and pestle. Ground flukes were rinsed from the mortar and pestle with 2 ml of RIPA buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 1% (v/v) Nonidet P-40, 0.1% (w/v) sodium deoxycholate, 0.05% (w/v) sodium dodecyl sulfate (SDS), 1% (v/v) Triton X-100). The FhTeg extract and FhW lysate were then centrifuged at 100,000*g* for 60 min. Supernatant was filtered through a 0.22 μm filter and dialysed overnight in RIPA dialysis buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5 mM EDTA, 0.05% (v/v) Nonidet P-40, 0.01% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100). Protein concentration was estimated using a Pierce™ BCA protein assay kit (Thermo Fisher Scientific, USA). FhTeg extracts were diluted to 250 μg/ml and FhW was diluted to 500 μg/ml with RIPA dialysis buffer.

2.2. LGALS-11 and LGALS-14 expression and purification

LGALS-11 and 14 were recombinantly expressed in *Escherichia coli* and purified as previously described (Sakthivel et al., 2015, 2018). Briefly, LGALS-11 and LGALS-14 were induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at O.D. 600 of 0.5 and further grown at 23 °C and 30 °C for 16 h, respectively. Cell pellets were lysed in 50 mM 4-(2-hydroxyethyl)-1-piperazinee thanesulfonic acid (HEPES) pH 8.8, 300 mM NaCl, 30 mM imidazole and 0.1% (v/v) Triton-X 100 with the addition of 5 μg/ml of DNAase, 10 μg/ml of lysozyme and 100 mM $MgCl_2$, for 60 min and then clarified by centrifugation at 18,000*g* for 25 min. The galectins were purified from the supernatant via nickel affinity chromatography and eluted with imidazole and with the addition of 20 mM tris(2-carbonexyethyl) phosphine (TCEP) following elution. Both galectins were further purified by size exclusion chromatography with a Hiload™ 16/600 Superdex 75 pg gel filtration column (GE Healthcare Life Science, USA) equilibrated in 20 mM HEPES pH 8.8, 100 mM NaCl and 5 mM TCEP using an ÄKTA Basic

fast protein liquid chromatography (FPLC) system. Purified LGALS-11 and LGALS-14 were concentrated to 1 mg/ml using Amicon Ultra Centrifugal filters (3 kDa molecular weight cut off; Millipore, Germany).

2.3. Conjugation of LGALS-11 and LGALS-14 onto N-hydroxysuccinimide (NHS)-activated sepharose

LGALS-11 and LGALS-14 were coupled to NHS-activated Sepharose (GE Healthcare Life Science) following the protocol provided by the manufacturer. Briefly, 1 volume of NHS-activated Sepharose was washed twice in 10 volumes of MilliQ water and activated in 10 volumes of ice cold 1 mM HCl. LGALS-11 and LGALS-14 were then added separately to the Sepharose beads, with the addition of HEPES to bring the final concentration to 50 mM HEPES. To allow for coupling of the galectins to the Sepharose beads, samples were mixed on an end over end mixer for 16 h at 4 °C followed by 2 h at room temperature. The remaining activated sites were blocked in 100 mM Tris-HCl pH 8.5, 500 mM NaCl and 10 mM TCEP for 3 h at room temperature. The coupled beads were then washed three times each with 10 volumes of two alternate wash buffers, wash buffer 1 (100 mM Tris-HCl pH 8.8, 500 mM NaCl and 10 mM TCEP) and wash buffer 2 (100 mM HEPES pH 6.8, 500 mM NaCl and 10 mM TCEP). Galectin conjugated beads were stored in storage buffer (100 mM Tris-HCl pH 8.0, 150 mM NaCl and 0.05% (w/v) sodium azide) until further use.

2.4. Capture of LGALS-11- and LGALS-14-specific parasite ligands

The three biological replicates of both FhTeg and FhW protein preparations were exposed to both LGALS-11 and LGALS-14 immobilised Sepharose beads using batch binding. FhTeg (100 μg) and FhW (350 μg) were incubated with 150 mg and 300 mg of galectin coupled Sepharose beads, respectively. Ligand binding was achieved at room temperature for 3.5 h with gentle agitation. The supernatant containing the unbound proteins was removed by centrifugation at 500*g* for 1 min. The bead-galectin-ligand complex was then washed three times by gently mixing the beads in RIPA dialysis buffer for 10 min, centrifugation as above and the supernatant removed each time. Galectin bound ligands were then eluted by incubating in 250 mM galactose, 20 mM Tris-HCl pH 8.0, 100 mM NaCl and 10 mM TCEP for 30 min. The two fluke preparations, pool of the three washes and eluted protein fractions were analysed by 12% (w/v) SDS-PAGE stained with silver nitrate as per the manufacturer's instructions (BioRad Silver Stain Plus, Australia).

2.5. Sodium meta-periodate treatment

To determine if the ligand binding observed was in fact a carbohydrate-protein interaction, aliquots of each fluke protein preparation (FhTeg and FhW) were treated with mild periodate oxidation as per the method of Schallig and van Leeuwen (1996) in order to disrupt the glycan structure but maintain protein integrity. Briefly, each sample was dialysed into PBS and 20 mM of sodium meta-periodate in 50 mM sodium acetate buffer (pH 4.5) and was incubated in the dark for 1 h at room temperature with agitation. Excess sodium meta-periodate was removed by adding an equal volume of ethylene glycol and incubating for 10 min followed by centrifugation at 10,000*g* for 10 min. The resulting pellet was discarded. To confirm complete glycan modification by sodium meta-periodate, a lectin blot was performed by separating periodate- and non-periodate-treated samples on a 12% (w/v) SDS-PAGE. Proteins were then either fixed and silver stained or transferred onto a polyvinylidene difluoride (PVDF) membrane and probed with horseradish peroxidase conjugated Concanavalin A (ConA-HRP) lectin (Sigma-Aldrich, USA). The sodium meta-periodate-treated FhTeg and FhW samples were batch bound with immobilised LGALS-11 and LGALS-14, washed and eluted with galactose as previously described. Mass spectrometry was conducted on the eluted fraction of these samples.

2.6. Mass spectrometry

Eluted protein samples were dried using a SpeedVac Concentrator and Savant Refrigerated Vapor trap (ThermoFisher) before resolubilisation in 100 μL of solution of 8 M Urea, 100 mM Tris-HCl pH 8.3. One microlitre of 200 mM TCEP was then added to the samples (and incubated overnight at 21 °C in a ThermoMixer (ThermoFisher). Four microlitres of 1 M iodoacetamide (IAA; in water) were added the following day and samples were incubated in the dark at 21 °C. Five hundred microlitres of 50 mM Tris-HCl (pH 8.3) and 1 μg of sequencing-grade trypsin (Promega, USA) were then added to samples and left overnight at 37 °C in an incubator. The digests were acidified with 1% (v/v) trifluoroacetic acid (TFA) and the peptides desalted on poly(styrene-divinylbenzene) copolymer (SDB) StageTips (3 M, USA) as described previously (Rappsilber et al., 2007).

Trypsin-digested peptides were reconstituted in 0.1% (v/v) TFA and 2% (v/v) acetonitrile (ACN) and then loaded onto a guard column (C_{18} PepMap 100 μm inner diameter (ID) × 2 cm trapping column, Thermo-Fisher Scientific) at 5 μl/min and washed for 6 min before switching the guard column in line with the analytical column (Vydac MS C_{18}, 3 μm, 300 Å and 75 μm ID × 25 cm). The separation of peptides was performed at 250 nl/min using a linear ACN gradient of buffer A (0.1% (v/v) formic acid, 2% (v/v) ACN) and buffer B (0.1% (v/v) formic acid, 80% (v/v) ACN), starting at 5% (v/v) buffer B to 30% in 65 min and then to 50% B at 78 min. The column was then eluted from peptides at 99% B for 5 min following equilibration at 5% B for 5 min. Data were collected on an Orbitrap Elite (ThermoFisher) in a data-dependent acquisition mode using m/z 300–1500 as MS scan range, collision-induced dissociation (CID) MS/MS spectra was collected for the 20 most intense ions. Dynamic exclusion parameters were set as described previously (Nguyen et al., 2016). The Orbitrap Elite was operated in dual analyser mode, with the Orbitrap analyser being used for MS and the linear trap being used for MS/MS. Pull-down and LC-MS/MS analysis were performed three times on different days.

2.7. Protein identification and quantification

Identification and label-free quantification (LFQ) of proteins were achieved using MaxQuant (version 1.16.0; (Cox and Mann, 2008)) together with its built-in search engine Andromeda. A protein database consisting of all predicted proteins from the FhD transcriptome which is publicly available on GenBank (GEVX01000000) (Cameron et al., 2017) and the bovine proteome (UniProt, February 2016) together with the common contaminants listed in MaxQuant, was used for this analysis. Briefly, carbamidomethylation of cysteines was set as a fixed modification, acetylation of protein N-termini, methionine oxidation were included as variable modifications. Precursor mass tolerance was 10 ppm, product ions were searched at 0.5 Da tolerances, minimum peptide length defined at 6, maximum peptide length 144, and peptide spectral matches (PSM) were validated using Percolator based on q-values at a 1% false discovery rate (FDR). Both peptide and protein identifications were reported at a FDR of 1%. The mass spectrometry proteomics data has been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with identifier PXD012787.

The protein group intensity values were obtained from MaxQuant and the LFQ intensity values were used to compare the

abundance of identified proteins between the FhW fractions enriched by both LGALS-11 and LGALS-14, as well as to investigate the abundance of individual proteins identified within the FhW LGALS-14 experiment alone. Proteins were only included in the dataset if those appeared in all three biological replicates and not in the sodium meta-periodate treatment dataset. A *t*-test was used to calculate the difference in abundance between proteins in the FhW fractions captured by LGALS-11 and LGALS-14. The *P* values obtained were corrected for multiple testing using the method of Benjamini and Hochberg (1995). $P < 0.5$ was considered significant. To compare the abundance of individual proteins within the FhW LGALS-14 experiment, LFQ intensity values were Log_2 transformed and quantile normalised. The mean of the three biological replicates was taken as the calculated abundance. This was completed using the *limma* package in R.

2.8. Analysis of glycosylation

NetNGlyc 1.0 Sever (http://www.cbs.dtu.dk/services/NetNGlyc/) was used to determine potential N-glycosylation sites using the default settings. Similarly, potential O-linked glycosylation sites were predicted using NetOGlyc 4.0 Sever (http://www.cbs.dtu.dk/services/NetOGlyc/).

2.9. Transcriptomic analysis of identified proteins

Genes identified following LGALS-11 and LGALS-14 capture assays were mapped to the published transcriptomic dataset (Cwiklinski et al., 2015a) and displayed as a heat map using R. The average of the transcript data for each developmental stage was normalised by log_{10} transforming the values and displayed as transformed transcripts per million reads (TPM). The life stages analysed included egg, metacercariae, newly excysted juvenile (1, 3, and 24 h post excystment), 21 days old juvenile (immature fluke) and adult fluke. The relative changes in transcript levels for each protein between the different life stages was *z*-score adjusted using the log_{10} transformed values and the equation ([TPM value – mean]/standard deviation). A colour scale of red (down-regulated) and yellow/white (up-regulated) was used to visualise the deviation from the average log_{10}TPM value (orange).

3. Results

3.1. LGALS-11 and LGALS-14 bound to F. hepatic glycoproteins in a carbohydrate-dependent manner

Lysates diluted to equal concentrations were visualised using a silver stain to ensure uniformity between the three replicates of FhTeg and FhW prior to batch binding to the galectins (Supplementary Fig. S1). Multiple bands were observed with a broad range of molecular weights, with little variation seen between the three replicates of FhTeg and FhW lysates. Following batch binding, washing with dialysis RIPA buffer and elution with 250 mM galactose, a pooled sample of the three washes (Fig. 1) was visualised and compared with the elution profile (Fig. 1). It is evident that during the elution step some of the recombinant LGALS-11 and LGALS-14 was also co-eluted, however, it did not appear in the wash solutions. Treating the lysates with mild sodium meta-periodate successfully altered the glycan structures as demonstrated by the treated lysates migrating very similarly through the gel, however not being recognised by the mannose-specific lectin ConA (Fig. 2). Additionally, following the parallel control glycoprotein capture, using sodium meta-periodate lysates, a total of 11 *F. hepatica* proteins were identified (Supplementary Table S1), all of which were then removed from the experimental dataset. The low number of proteins identified indicates that LGALS-11 and LGALS-14 are interacting with proteins in a carbohydrate-dependent manner.

3.2. LGALS-11 and LGALS-14 bound to F. hepatic glycoproteins with a range of functions and subcellular locations

Fasciola hepatica glycoproteins which were captured by LGALS-11 and LGALS-14 were eluted by the additions of galactose, which is an inhibitory sugar of galectin and identified by mass spectrometry (Supplementary Table S2). In total, LGALS-14 interacted with 255 *F. hepatica* proteins; of these, 44 were found in the tegument extract, but only one was specific to the tegument extract with 211 proteins specific to the FhW lysate (Fig. 3A). Strikingly different results were obtained from the LGALS-11 pull-down experiments with only 49 *F. hepatica* proteins identified, eight of which were identified in both the FhTeg and the FhW extracts, with no proteins specific to the FhTeg samples (Fig. 3b). Of these 49 proteins, all were also represented in the LGALS-14 fractions (Fig. 3C). Although these proteins were present in the LGALS-11 eluted fractions, by comparison of the total abundance and the observed fold change between the means of the LGALS-11 and the LGALS-14 fractions, it is clear that they were all far more abundant in the LGALS-14 fractions (Fig. 4).

The predicted subcellular location and function of these proteins is illustrated in Fig. 5, showing great diversity. The two functional categories of cell structure and proteins with unknown functions contained the greatest number of individual proteins equating to 9.80 and 9.02% of the total dataset, respectively (Fig. 5A). Protein folding and transporter proteins closely followed, with both making up 8.63% of the data set. The subcellular location of the 255 proteins was predicted based on consideration of the number of transmembrane domains, gene ontology (GO) terms, as well as comparing the location of homologous proteins within UniProt. Additionally, proteins were assigned a predicted membrane location if they were identified in the peripheral or integral membrane fractions of the study conducted by Wilson et al. (2011). By doing so, it was determined that the 255 protein were made up of 22.35% cytoplasmic, 13.33% cytoskeletal, 16.06% membrane, 22.35% mitochondrial, 5.10% endoplasmic reticulum (ER), 7.84% secreted, 1.96% lysosomal, 1.57% nuclear/cytoplasmic, 0.39% peroxisome/ER, 1.18% nuclear and 2.75% ribosomal, with 5.10% of proteins which could not be assigned a predicted location (Fig. 5B).

The number of possible N- or O-glycosylation sites were predicted for the 255 proteins identified. Only 15 proteins had no predicted glycosylation site (Supplementary Table S2). These 15 non-glycosylated proteins included five natterins, six mitochondrial proteins, two twitchin proteins and fatty acid binding protein 3 (FABP3). However, of the 240 proteins that contained potential glycosylation sites, only 136 of those proteins contained an ER signal peptide. This could partially be attributed to incomplete sequence data which may produce truncated predicted protein sequences, hence no signal peptides were identified.

The protein that was evidently the most abundant protein in all of the LGALS-11 and LGALS-14 fractions was an uncharacterised protein that contained a C-type lectin domain (FhD34851) (Table 1, Supplementary Table S2). Another family of proteins that was surprisingly more abundant than other proteins is the natterins; in total, six natterins were identified, five of which belong to the natterin-4 family and one to the natterin-3 family. Many proteins that have been trialled previously for vaccine candidates have also been identified and these include glutathione S-transferases (GST), paramyosin, cathepsin L, FABP3 and leucine aminopeptidases (LAP) (Table 1, Supplementary Table S2).

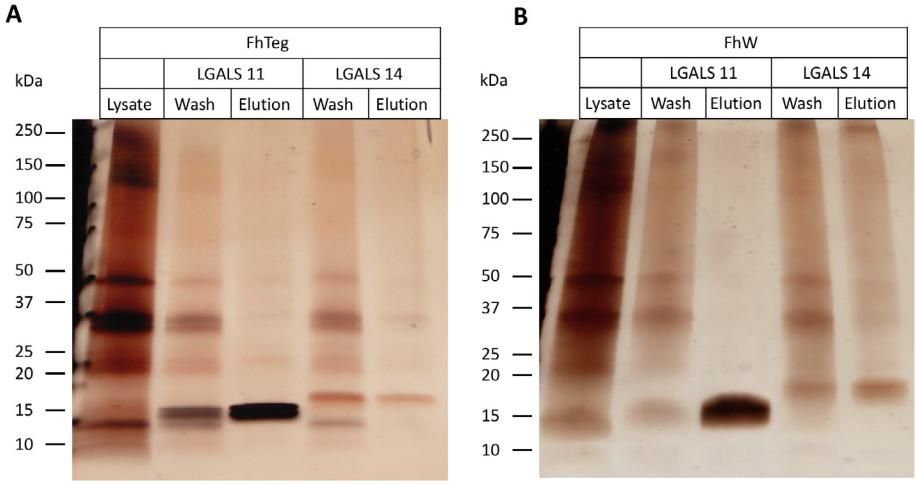

Fig. 1. Protein profile of *Fasciola hepatica* tegument (A) and whole worm (B) extracts bound to galectin-11 (LGALS-11) and galectin-14 (LGALS-14). Lysate material was added to immobilised LGALS-11 and LGALS-14, washed and bound; glycoproteins were eluted with 250 mM galactose.

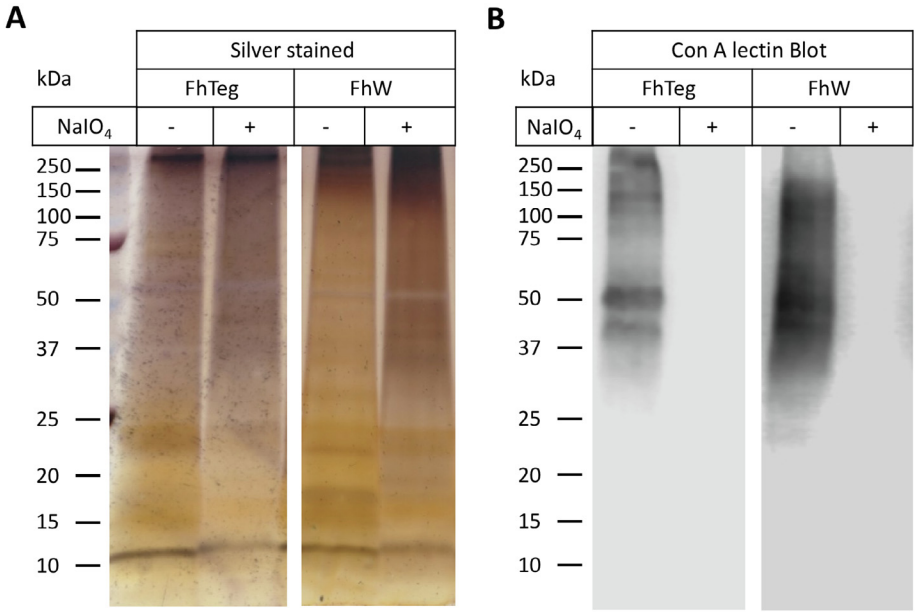

Fig. 2. Silver stained SDS-PAGE gels and ConA lectin blot confirming successful glycan disruption by sodium periodate treatment of *Fasciola hepatica* tegument protein extracts and whole worm protein extract. (A) A comparison of the non-treated protein profile (−) and sodium meta-periodate-treated protein profile (+) for each FhTeg and FhW extract was separated by SDS-PAGE and silver stained. (B) An identical gel was transferred to a nitrocellulose membrane and probed with horseradish peroxidase conjugated Concanavalin A to ensure complete glycan disruption of each of the FhTeg and FhW proteins.

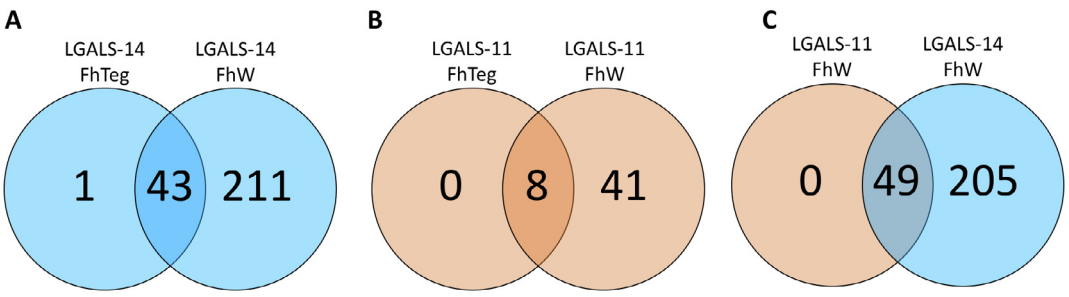

Fig. 3. Venn diagram of *Fasciola hepatica* tegument extract and the whole worm proteins bound by host galectins. The distribution of proteins bound by galectin-14 (LGALS-14) (A) and galectin-11 (LGALS-11) (B). In larval and adult stages, 0 and 26 proteins were bound by both the galectins, respectively.

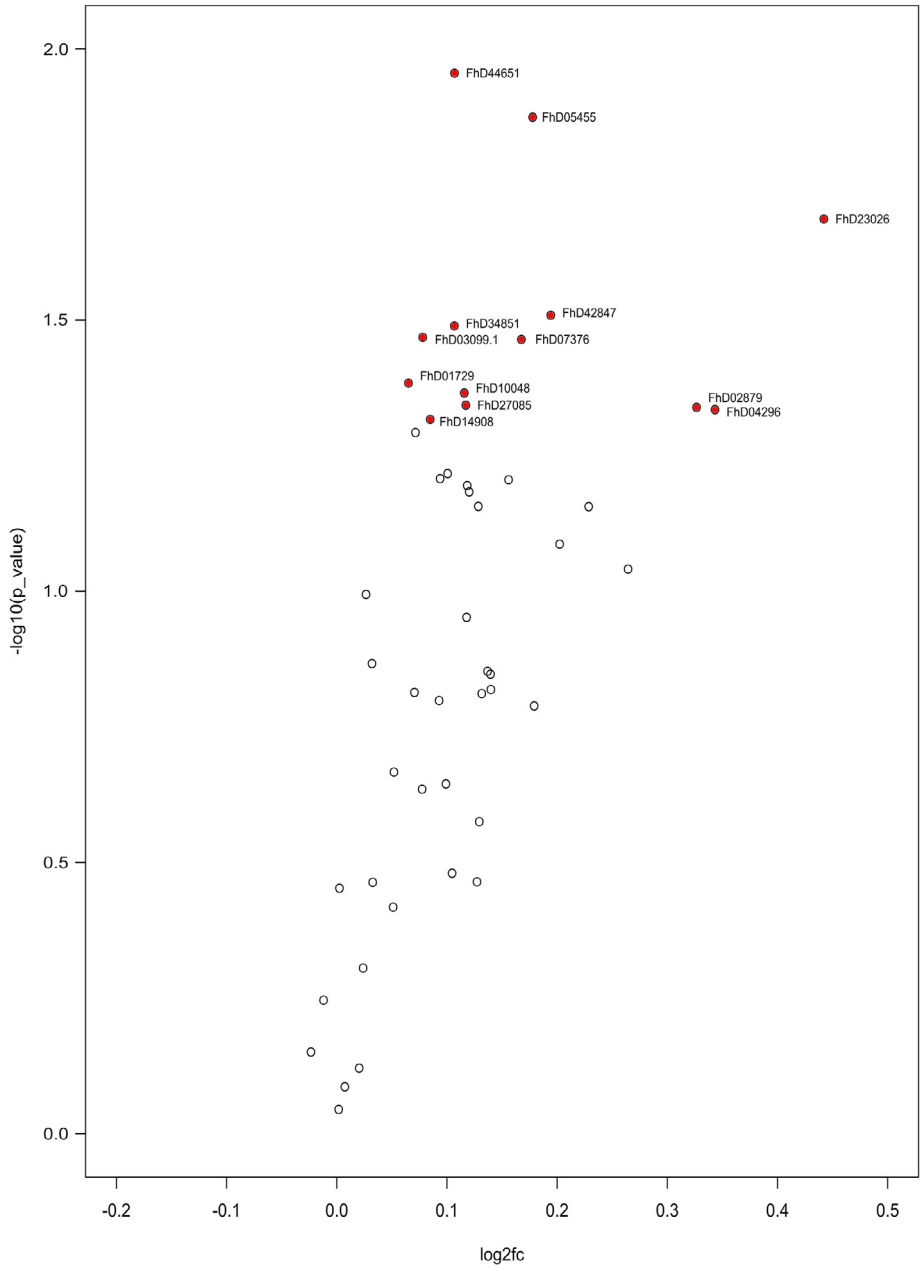

Fig. 4. Volcano plot showing the fold change increase ($\log_2$ fold change) against the $\log_{10}$ *P* value of individual *Fasciola hepatica* proteins identified in the whole worm extract, that was eluted from immobilised galectin-11 (LGALS-11) and galectin-14 (LGALS-14). Red labelled dots represent proteins with a significant difference in abundance when comparing the abundance of these proteins after enrichment by LGALS-14 with LGALS-11 ($n = 13$). A positive fold change represents a higher protein abundance in the LGALS-14 compared with the LGALS-11 binding of the same lysates.

3.3. Protein transcript expression pattern during fluke development

Using the RNAseq dataset published by Cwiklinski et al. (2015a), the transcript expression level associated with each protein was determined across each of the *F. hepatica* life stages from metacercaria to egg (Fig. 6, Supplementary Fig. S2). The majority of the proteins showed an up-regulation of transcript expression within the adult life stages; however, some were downregulated within the adult life stage, demonstrating that this method did not only target highly abundant proteins. Of the proteins that are downregulated in adult flukes, most were identified as T-complex proteins, transcriptional-associated proteins and mitochondrially localised proteins. The most abundant protein identified in the galectin assay was the uncharacterised C-type lectin and this protein has moderate transcript levels during the life stages from metacercaria through to immature fluke and much higher transcript levels during the egg and the adult stages of life.

4. Discussion

The surface of *F. hepatica* is protected by a complex glycocalyx made up of glycoproteins and glycolipids resulting in glycans having direct contact with the host immune system (Threadgold, 1976). Therefore, it is predicted that host lectins are likely involved in parasite surveillance and innate immunity (Prasanphanich et al., 2013). Galectins are a family of lectins which are categorised broadly as recognising N-acetyllactosamine and act as pattern

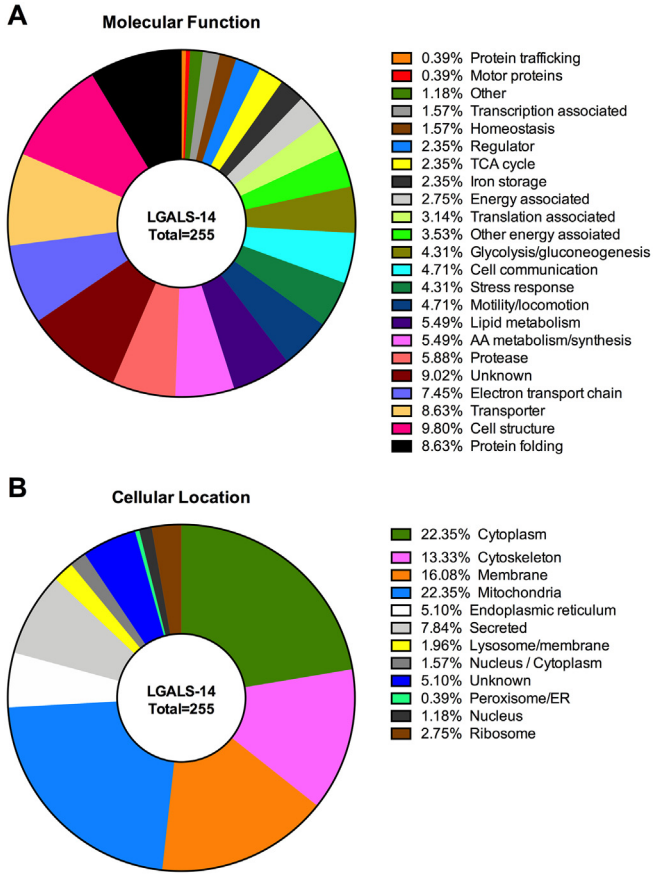

Fig. 5. Categorization of proteins in the tegument and whole worm extract of *Fasciola hepatica* that interacted with host galectin-14 (LGALS-14). The profiles were categorized based on biological processes of LGALS-14 bound-proteins (A) and cellular location of LGALS-14-bound proteins (B). AA, amino acid; ER, endoplasmic reticulum; TCA cycle, tricarboxylic acid cycle.

recognition receptors (PRR) in order to activate innate and adaptive immune responses following detection of pathogens (Rabinovich and Toscano, 2009). Two galectins previously associated with the presence of parasitic helminths in ruminants are LGALS-11 and LGALS-14 (Dunphy et al., 2000, 2002). This study shows, to our knowledge for the first time, specifically which *F. hepatica* glycoproteins LGALS-11 and LGALS-14 are interacting with and compares the abundance of these proteins identified by both.

In total, LGALS-11 interacted with 49 *F. hepatica* proteins. These same 49 proteins were all also shown to interact with LGALS-14. However, when the protein abundance is compared between the proteins interacting with the two galectins it is clearly evident that LGALS-14 has a far larger number of binding partners. This suggests that LGALS-14 is interacting directly with *F. hepatica*, while it is possible that LGALS-11 is not. This result is supported by work conducted by Young et al. (2012) who observed a lack of recombinant LGALS-11 binding to mounted longitudinal cross-sections of *F. hepatica,* compared with recombinant LGALS-14 binding to the complete outer surface of cross-sections prepared the same way. Both these results are in contrast to similar studies carried out with the gastrointestinal nematode *H. contortus*, which shows LGALS-11 binding to the pharynx and rectal region of L4s and the surface of adults (Preston et al., 2015a). Recently, Sakthivel et al. (2018), completed a similar experiment to the one conducted here, whereby L4 and adult *H. contortus* lysates were passed over corresponding galectin columns. This demonstrated that LGALS-11 did not interact with any L4 larval glycoproteins but it did interact with 32 adult *H. contortus* proteins. The reason there could be possible discrepancies between these *H. contortus* studies could be explained by the hypothesis that LGALS-11 is interacting with glycolipids rather than glycoproteins. Alternatively, and more strongly supported in regard to *Fasciola*-galectin interactions, is that LGALS-11 has evolved to interact with host ligands rather than parasite ligands in order to manipulate the parasite's environment as part of the host immune response. Up-regulation of LGALS-11 upon infection with *H. contortus* has been correlated with an increase in intestinal mucosa "stickiness" (Dunphy et al., 2000; Robinson et al., 2011). The difference in LGALS-11 recognition of *F. hepatica* and *H. contortus* is not unexpected, as other galectins have also been shown to have differing abilities to recognise different parasite species even of the same genus; for example, galectin-3 interacts with *Leishmania major* but not *Leishmania donovani* (Pelletier and Sato, 2002). Evidently, the specific role of LGALS-11 in the host response to *F. hepatica* infection warrants further investigation.

The observation of LGALS-14 binding to a high number of *F. hepatica* glycoproteins is not all that surprising considering it is known to interact with a low number of repeats of LacNAc (Galβ1-4GlcNac; N-acetyllactosamide) (Young et al., 2009). Recently, LacNAc has been shown to be a terminal antenna of the glycans displayed on the tegument of adult *F. hepatica* (Ravidà et al., 2016a). The most abundant protein in the LGALS-14 fractions was an uncharacterised protein which contains a C-type lectin domain (FhD34851). This particular protein is predicted to be highly glycosylated as the sequence contains a signal peptide, one potential N-glycosylation site and 65 potential O-glycosylation sites. LGALS-14 contains one potential N-glycosylation site, but as this protein was expressed in an *E. coli* expression system the recombinant LGALS-14 could not be glycosylated (Sahdev et al., 2008). Additionally, galectins are secreted through the unconventional secretion pathway and therefore bypass the ER and Golgi where glycosylation occurs (Popa et al., 2018). Therefore, we are confident that this protein was identified as a result of direct galectin-glycan interaction. This protein has been reported in the peripheral or integral membrane fractions by Wilson et al. (2011) and extracellular vesicle (EV) preparations by Cwiklinski et al. (2015b). FhD34851 also has 57.1% homology with the *Schistosoma mansoni* protein Smp_149610 (described as a hypothetical protein) which contains a serine and threonine-rich mucin-like region and a C-type lectin domain (Fitzpatrick et al., 2009). Transcript data shows that this uncharacterised *F. hepatica* C-type lectin is mainly transcribed during the egg and adult life stages, so although it is desirable to have a drug or vaccine which targets newly excysted juveniles (Toet et al., 2014), if this protein is highly immunogenic as is suggested by its interactions with both LGALS-11 and LGALS-14, it could be a potential candidate to investigate further.

The only protein identified that was unique to the LGALS-14/FhTeg fraction was a 14-3-3 protein (FhD18047). Unfortunately, the tegument extract did not contain as many unique proteins as anticipated and evidently contained many internal proteins compared with the FhW fraction due to the simple sodium deoxycholate detergent extraction method implemented. Nonetheless, the 14-3-3 family of proteins has a diverse repertoire of functions and is thought to play a role in host-parasite interactions (Andrade et al., 2004). In trematodes, it has been shown to be expressed within the reproductive organs, tegument, sub-tegumental layer and parenchyma (Schechtman et al., 2001b; Chaithirayanon et al., 2006). Recently, these proteins have been used as antigens to vaccinate against many parasites including *S. mansoni*, *S. japonicum*, *Trichinella spiralis*, *Echinococcus multilocularis* and recently *F. hepatica* (Schechtman et al., 2001a; Pérez-Caballero et al., 2018). Isoforms of 14-3-3 have also been studied in *Toxoplasma gondii* and *F. gigantica*. In this dataset, two gamma (FhD18524 and FhD18047) and one zeta (FhD26570) *F. hepatica*

Table 1
Identification by mass spectroscopy of top 50 *Fasciola hepatica* proteins eluted from a galectin-14 column.

FhD number	Accession number	Protein annotation	Mol. weight (kDa)	Sequence length	Unique peptides	Sequence coverage (%)	Average abundance	Number of predicted glycosylation sites		Signal peptides	Cellular location	Function
								N-Glycosylation	O-Glycosylation			
FhD34851	GEVX01028574.1	Uncharacterised C-type lectin	47.82	441	26	38.8	27.23	1	65	✓	Secreted/ extracellular	Unknown
FhD01961	GEVX01001811.1	Myosin heavy chain, striated muscle	223.19	1946	106	51.8	25.73	13	51	x	Cytoskeleton	Cell structure
FhD03099	GEVX01002866.1	Probable succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	42.51	392	18	65.3	25.09	0	4	x	Mitochondria	ETC involvement
FhD09193	GEVX01008502.1	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	42.61	384	7	68.8	24.90	1	3	x	Mitochondria	ETC involvement
FhD10048	GEVX01009183.1	Tubulin beta chain	50.66	452	4	57.5	24.50	3	0	x	Cytoskeleton	Major component of microtubules
FhD36869	GEVX01030120.1	Natterin-4	16.83	155	10	90.3	24.00	0	0	x	Membrane	Likely hydrolase
FhD07376.1	GEVX01006835.1	Propionyl-CoA carboxylase alpha chain, mitochondrial	62.75	565	34	69.9	24.00	0	1	x	Mitochondria	Fatty acid catabolism
FhD35489	GEVX01029069.1	Paramyosin	99.42	865	40	50.5	23.97	4	44	x	Cytoskeleton	Cell structure
FhD26501	GEVX01022139.1	Natterin-4	16.44	155	0	98.1	23.86	0	0	x	–	Unknown
FhD44651	GEVX01036000.1	2-Oxoglutarate dehydrogenase, mitochondrial	115.60	1025	0	39.8	23.54	3	17	x	Mitochondria	TCA cycle (energy)
FhD27085	GEVX01022586.1	Heat shock cognate 71 kDa protein	72.82	662	10	32.2	23.47	6	8	✓	Cytoplasm	Chaperone stress response
FhD42847	GEVX01034613.1	ATP synthase subunit beta, mitochondrial	58.25	544	21	57.5	23.45	0	7	x	Mitochondria	ETC
FhD02879	GEVX01002658.1	Transforming growth factor-beta-induced protein ig-h3	85.54	755	29	51.9	23.28	1	12	✓	Secreted/ extracellular	Adhesion
FhD45620	GEVX01036722.1	L-Threonine dehydratase catabolic TdcB	49.79	464	15	34.9	23.21	0	4	x	Cytoplasm	Amino acid catabolism
FhD03038	GEVX01002809.1	Choline transporter-like protein 2	84.58	747	11	19.7	23.04	3	2	x	Membrane	Exocytosis
FhD26453	GEVX01022100.1	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	32.35	278	19	58.6	22.96	2	5	✓	Mitochondria	Involved in electron transport chain
FhD43431	GEVX01035058.1	Ferritin, middle subunit	26.39	228	3	57.9	22.89	1	6	x	Likely cytoplasm	Iron transport
FhD44990	GEVX01036254.1	Heat shock protein HSP 90-alpha 1	54.77	471	20	44.2	22.48	2	5	x	Cytoplasm	Chaperone stress response
FhD23026	GEVX01019438.1	Periostin	71.12	631	20	42.8	22.47	2	9	x	Secreted/ extracellular	Adhesion
FhD42604	GEVX01034439.1	Tubulin alpha-1A chain	49.03	442	6	45.2	22.34	1	1	x	Cytoskeleton	Major component of microtubules
FhD44990.1	GEVX01036254.1	Heat shock protein HSP 90-alpha	31.02	277	11	44	22.33	2	5	x	Cytoplasm	Chaperone stress response
FhD04296	GEVX01003973.1	Alpha-aminoadipic semialdehyde synthase, mitochondrial	96.61	859	29	48.5	22.15	5	6	x	Mitochondria	Amino acid catabolism
FhD03116	GEVX01002883.1	Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial	56.61	516	14	36.6	22.11	1	23	x	Mitochondria	Fatty acid synthesis, ketone body
FhD12787	GEVX01011308.1	Fructose-1,6-bisphosphatase 1	36.80	338	10	50.6	22.00	1	2	x	Cytoplasm	Gluconeogenesis
FhD01235	GEVX01001152.1	Glyceraldehyde-3-phosphate dehydrogenase	37.81	351	19	65.5	21.93	1	6	x	Cytoplasm	Glycolysis
FhD33768	GEVX01027737.1	Sodium/potassium-transporting ATPase subunit alpha	116.34	1051	13	28.3	21.86	3	0	x	Plasma membrane	Ion transport

Table 1 (*continued*)

FhD number	Accession number	Protein annotation	Mol. weight (kDa)	Sequence length	Unique peptides	Sequence coverage (%)	Average abundance	Number of predicted glycosylation sites		Signal peptides	Cellular location	Function
								N-Glycosylation	O-Glycosylation			
FhD07513	GEVX01006963.1	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	47.48	435	12	37.2	21.74	0	18	x	Mitochondria	Pyruvate DH complex - energy
FhD42994	GEVX01034729.1	Tropomyosin-1	32.82	284	12	57.4	21.47	0	8	x	Cytoskeleton	Muscle contraction cytoskeletal
FhD04241	GEVX01003919.1	Putative aminopeptidase W07G4.4	49.41	456	8	42.5	21.47	2	3	x	Secreted	Peptidase
FhD31658	GEVX01026107.1	Severin	41.75	366	20	49.5	21.46	0	5	x	Cytoskeleton	Villin/Gelsolin
FhD33863	GEVX01027816.1	2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial	47.76	424	14	43.4	21.24	3	4	x	Mitochondria	TCA cycle (energy)
FhD23894	GEVX01020121.1	Four and a half LIM domains protein 2	63.71	562	14	39	21.24	1	18	x	Cytoplasm/ Nucleus	Regulator
FhD04020	GEVX01003712.1	Carbonic anhydrase 2	34.84	314	8	29.9	21.19	5	6	✓	Cytoplasm/ Membrane	Transport associated
FhD02966	GEVX01002739.1	Sideroflexin-1	35.84	324	10	32.1	21.16	2	0	x	Mitochondrion membrane	Iron transport
FhD07280	GEVX01006742.1	Spectrin alpha chain	282.59	2432	21	13	21.09	6	96	x	Cytoskeleton	Cell structure
FhD14908	GEVX01012994.1	Glutathione S-transferase class-mu 28 kDa isozyme	24.96	219	8	36.1	21.09	0	3	x	Secreted	Free radical prevention, detoxification
FhD01935.1	GEVX01001787.1	Retinal dehydrogenase 1	42.90	401	9	25.2	21.09	1	0	x	Cytoplasm	Other energy associated
FhD05482	GEVX01005077.1	Chymotrypsinogen B	56.43	502	10	21.3	21.03	4	19	✓	Secreted	Protease
FhD11309	GEVX01010160.1	Natterin-4	18.46	174	0	78.7	20.98	0	0	x	–	Unknown
FhD03667	GEVX01003392.1	Prohibitin-2	30.39	275	9	45.1	20.95	1	1	x	Mitochondria	Inhibits DNA synthesis
FhD04455	GEVX01004117.1	NADP-dependent malic enzyme	63.74	584	17	47.1	20.74	2	3	x	Cytoplasm	Malate metabolism
FhD07376.1	GEVX01006835.1	Propionyl-CoA carboxylase alpha chain, mitochondrial	20.72	191	5	42.9	20.71	0	4	x	Mitochondria	ETC involvement
FhD28726	GEVX01023873.1	Methylmalonyl-CoA mutase, mitochondrial	46.78	429	13	32.2	20.68	0	4	x	Mitochondria	Fatty acid catabolism
FhD39034	GEVX01031736.1	Voltage-dependent anion-selective channel protein 2	31.37	279	7	29.4	20.65	0	0	x	Mitochondrial membrane	Transporter
FhD30107	GEVX01026146.1	Cathepsin L-like proteinase	25.83	233	9	51.1	20.54	0	1	x	Secreted	Protease
FhD35538	GEVX01029105.1	Oxalate:formate antiporter	52.29	481	5	8.9	20.53	1	0	x	Mitochondria	Links glycolysis and TCA cycle
FhD06912	GEVX01006398.1	Annexin A11	51.58	472	10	25.4	20.52	0	6	x	Membrane	Exocytosis
FhD02765	GEVX01002554.1	Histone H2B.3	15.38	138	4	19.6	20.52	0	9	x	Nucleus	Transcription regulation
FhD23858	GEVX01020097.1	Tropomyosin-2	32.76	284	13	61.6	20.51	1	16	x	Cytoskeleton	Muscle contraction
FhD41034	GEVX01033244.1	Annexin A13	29.44	260	5	62.3	20.46	0	3	x	Membrane	Exocytosis

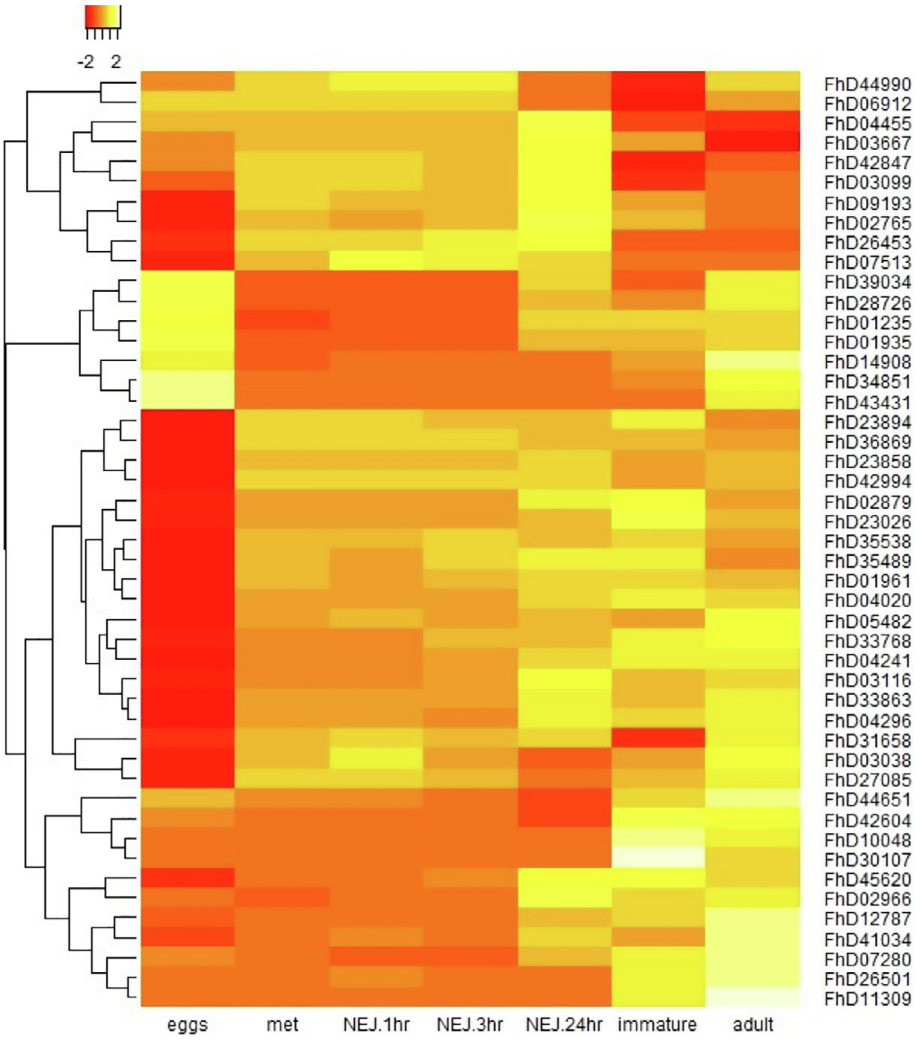

Fig. 6. Heat map illustrating RNAseq expression levels across definitive host life stages of *Fasciola hepatica* for the transcripts associated with the top 50 most abundant proteins identified to bind to host galectin-14 (LGALS-14). The heatmap is coloured using the *z*-score for each individual row, with orange representing a *z*-score of 0, indicating the mean value for that row, red colouration indicating levels below the mean transcript level and yellow to white indicating above average transcript levels for that life stage.

14-3-3 proteins were identified. Recently, recombinant Fh14-3-3 zeta (FhD26570) was used to vaccinate experimentally infected sheep with no significant levels of protection observed; however, infected sheep sera did not recognise the recombinant protein (Pérez-Caballero et al., 2018). Conversely, when the effects of an *F. gigantica* 14-3-3 epsilon protein (with 100% identity to another *F. hepatica* 14-3-3 protein) was presented to goat peripheral blood mononuclear cells (PBMCs) it was shown to bind to the surface of these host cells and induce the production of the cytokines IL-10 and TGF-β in vitro, while also inhibiting cell proliferation, reducing phagocytosis and stimulating apoptosis of PBMCs, suggesting that this protein plays a role in parasite survival mechanisms (Tian et al., 2018).

One group of proteins that had a much higher abundance in the eluted fractions contained six proteins belonging to the natterin family, characterised as having at least one DM9 domain. Natterins share homology with enzymes that cause vasodilation and oedema when secreted in the venom of the fish *Thalassophryne nattereri* (Magalhães et al., 2005). It has previously been speculated that a protein of this nature would be beneficial for a blood feeding parasite (Haçarız et al., 2014). Although the exact function of this family of *F. hepatica* proteins is unknown, they are thought to play a role in vesicular transport, nutritional uptake and immune invasion (Gaudier et al., 2012; Phadungsil et al., 2016). Interestingly, the natterin proteins identified here are expressed at the highest level during the juvenile life stage (24 h post excystment) (Cwiklinski et al., 2015a) supporting the previously suggested functions. Natterin-3 was identified by LGALS-14 in the FhTeg and FhW fractions; this protein has been recognised previously as a component of the *F. hepatica* tegument and extracellular vesicles (Wilson et al., 2011; Marcilla et al., 2012; Haçarız et al., 2014; Cwiklinski et al., 2015b; Cameron et al., 2017). Similarly, five individual natterin-4 proteins (FhD11309, FhD26501, FhD30312, FhD26064 and FhD36869) were identified in the present study, three of which have been identified in previous tegument and glycoprotein studies (FhD11309, FhD26501 and FhD36869) (Ravidà et al., 2016b; Wilson et al., 2011). Two Pacific oyster (*Crassostrea gigas*) proteins, which contain a duplicated DM9 domain (CgDM9CP-1 and CgDM9CP-2) and are believed to play a role in innate immunity as a pattern recognition receptor; these share 28.99–32.61% sequence identity with the six natterin proteins identified in this study. Both these oyster proteins have been shown to have undescribed lectin activity with high specificity and avidity to the monosaccharide D-mannose, as well as high mannose-type N-glycans and mannosylated bi- or tri-antennary hybrid oligosaccharides (Unno et al., 2016; Jiang et al., 2017; Liu

et al., 2018). *Fasciola hepatica* is known to contain many high mannose-type N-glycans (Garcia-Campos et al., 2016; Ravidà et al., 2016a) and due to the strong binding capacity of these *C. gigas* homologs, it is possible that the *F. hepatica* natterins are being captured as a consequence of their active interactions with *F. hepatica* mannosylated glycoproteins which have been enriched by the two galectins. None of the natterin proteins contain any predicted N-glycosylation or O-glycosylation sites, therefore it is unlikely to be a direct galectin-natterin interaction. Similarly, FABP3 is another protein which does not contain any potential glycosylation sites but has appeared in both the present glycoprotein study and one previously described glycoprotein study of *F. hepatica* (Ravidà et al., 2016b). Again, it is likely that this protein is consequently being captured indirectly due to its interaction with other proteins that are binding to the galectins.

An array of previous vaccine candidates appear in this dataset, including FABP, LAP, GST and paramyosin. As for the other vaccine candidates, they have had varying efficacies in previous vaccine trials. FABP had the highest efficacy in cattle with 55% protection (Hillyer et al., 1987) and, possibly due to the lack of predicted glycosylation sites, recombinant FABP (rFABP) showed similar limited protection (López-Abán et al., 2007). Two GST proteins were identified (FhD09197 and FhD14908); much research has been performed examining the function of GST, due to its role in immune invasion, by acting as an antioxidant enzyme and providing protection from free radicals (Piedrafita et al., 2007). Native GST has been trialled in multiple vaccine trials with varying protective success (Toet et al., 2014). Previously, Garcia-Campos et al. (2016) showed that one of the GST isoforms is glycosylated (most likely O-glycosylated). Recombinant GST has also been trialled, however it showed no levels of protection (De Bont et al., 2003; Kumar et al., 2012). It is possible that the glycan structures of the native proteins are essential components, potentially allowing correct conformation and/or aiding in antigenicity. As reviewed by Toet et al. (2014), it was emphasised that tegument proteins and glycoproteins are prime vaccine candidates due to their direct contact with the host immune system and are therefore targets for antibody-dependent cell cytotoxicity (ADCC) (Piedrafita et al., 2001, 2007). The importance of tegument proteins such as annexins, tetraspanins and CD59 proteins was highlighted and many of these proteins were identified in this dataset, including other tegument-specific proteins. Their presence within the present study indicates a potential role in triggering of adaptive immunity, possibly through direct interaction with LGALS-14 following secretion from eosinophils which have infiltrated the site of infection.

It has been shown that galectin-11 is upregulated and is involved in eliciting a protective response against *H. contortus* infection in studies comparing resistant and susceptible native Canary Island sheep (Guo et al., 2016). In the future, it would be interesting to investigate whether the interaction of LGALS-14 with *F. hepatica* is essential to eliciting a similarly protective effect. Currently, there are no host species that shows any resistance to *F. hepatica* infection except partially in rats and cows (Piedrafita et al., 2007). However, Indonesian thin tail sheep are resistant to *F. gigantica* and it would of interest to see if LGALS-14 is upregulated in these sheep upon infection (Roberts et al., 1997; Piedrafita et al., 2007).

Galectins are thought to play a role in regulating inflammatory responses to prevent collateral tissue damage and these processes can be taken advantage of by tumours (Rabinovich and Toscano, 2009); a similar scenario could be occurring with regard to *F. hepatica* infection, as *F. hepatica* has previously been suggested to hijack eosinophils which produce LGALS-14, as an immune evasion mechanism (Young et al., 2012; Preston et al., 2015b). Previously it has been reported that mouse macrophages infected with *Trypanosoma cruzi* have been shown to secrete galectin-1 (in low concentrations), which in turn blocks IL-12 secretion, resulting in enhanced parasite replication, as well as causing apoptosis of certain types of T cells (Zúñiga et al., 2001). Further investigation is needed into the role galectin-14 in the ruminant immune response to *F. hepatica* infection.

For the first known time, host-specific lectins were used to identify *F. hepatica* glycoproteins rather than generic lectins. The two lectins, LGALS-11 and LGALS-14, are both believed to play roles in the immune response of ruminants. It was found that LGALS-14 interacts with many proteins, with the most abundant being an uncharacterised protein that contains a C-type lectin domain and a serine threonine-rich C-terminus. In conclusion, this study has broadened the knowledge of the host-parasite interface with regard to *F. hepatica* infection, as well as identifying novel candidates for future study in parasite control strategies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.06.007.

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