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Proteomic identification of Galectin-11 and 14 ligands from *Haemonchus contortus*

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Haemonchus contortus is the most pathogenic nematode of small ruminants. Infection in sheep and goats results in anaemia that decreases animal productivity and can ultimately cause death. The involvement of ruminant-specific galectin-11 (LGALS-11) and galectin-14 (LGALS-14) has been postulated to play important roles in protective immune responses against parasitic infection; however, their ligands are unknown. In the current study, LGALS-11 and LGALS-14 ligands in *H. contortus* were identified from larval (L4) and adult parasitic stages extracts using immobilised LGALS-11 and LGALS-14 affinity column chromatography and mass spectrometry. Both LGALS-11 and LGALS-14 bound more putative protein targets in the adult stage of *H. contortus* (43 proteins) when compared to the larval stage (2 proteins). Of the 43 proteins identified in the adult stage, 34 and 35 proteins were bound by LGALS-11 and LGALS-14, respectively, with 26 proteins binding to both galectins. Interestingly, hematophagous stage-specific sperm-coating protein and zinc metalloprotease (M13), which are known vaccine candidates, were identified as putative ligands of both LGALS-11 and LGALS-14. The identification of glycoproteins of *H. contortus* by LGALS-11 and LGALS-14 provide new insights into host-parasite interactions and the potential for developing new interventions.

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19 # Abstract

20 *Haemonchus contortus* is the most pathogenic nematode of small ruminants. Infection in sheep
21 and goats results in anaemia that decreases animal productivity and can ultimately cause death.
22 The involvement of ruminant-specific galectin-11 (LGALS-11) and galectin-14 (LGALS-14) has
23 been postulated to play important roles in protective immune responses against parasitic
24 infection; however, their ligands are unknown. In the current study, LGALS-11 and LGALS-14
25 ligands in *H. contortus* were identified from larval (L4) and adult parasitic stages extracts using
26 immobilised LGALS-11 and LGALS-14 affinity column chromatography and mass spectrometry.
27 Both LGALS-11 and LGALS-14 bound more putative protein targets in the adult stage of *H.*
28 *contortus* (43 proteins) when compared to the larval stage (2 proteins). Of the 43 proteins
29 identified in the adult stage, 34 and 35 proteins were bound by LGALS-11 and LGALS-14,
30 respectively, with 26 proteins binding to both galectins. Interestingly, hematophagous stage-
31 specific sperm-coating protein and zinc metalloprotease (M13), which are known vaccine
32 candidates, were identified as putative ligands of both LGALS-11 and LGALS-14. The
33 identification of glycoproteins of *H. contortus* by LGALS-11 and LGALS-14 provide new
34 insights into host-parasite interactions and the potential for developing new interventions.

Introduction

Haemonchus contortus is a dominant blood feeding gastrointestinal nematode (GIN) parasite of small ruminants. Blood feeding by *H. contortus* results in haemorrhagic gastritis, oedema, diarrhoea and death in severe infections, leading to significant economic impact through decreased livestock production (Mavrot et al. 2015; McLeod 1995; Roeber et al. 2013). Sheep can develop effective immunity to *H. contortus* infection and vaccine-induced protection using *H. contortus*-derived molecules has been demonstrated, suggesting that the control of this parasite through vaccination is possible (Nisbet et al. 2016). However what host molecules recognise these glycoproteins are poorly understood. Recently it has been shown that galectins have been showed to play major roles in host defence against microbial pathogens. Galectins are a family of carbohydrate-binding molecules with characteristic domain organization and affinity for β-galactosides mediate a variety of important cellular functions, including inflammation and immune responses due to binding both self and non- self-glycans.

In particular, ruminants highly upregulate two specific galectins (LGALS-11 and LGALS-14 upon infection by various parasites such as *Ostertagia, Cooperia* and *H. contortus* (Dunphy et al. 2000; Dunphy et al. 2002; Hoorens et al. 2011; Meeusen et al. 2005). LGALS-14 is secreted by eosinophil immune cells that are critical for immunity through killing the larval stages of *H. contortus* (Balic et al. 2006; Dunphy et al. 2002; Young et al. 2009). LGALS-14 is thought to be the homologue of human galectin-10, which is also secreted by eosinophils (Ackerman et al. 2002). Analysis of *H. contortus* infected sheep demonstrated release of LGALS-14 into the gastrointestinal mucus, the interface of host and parasite interaction (Dunphy et al. 2002). In addition, kinetic studies of LGAL-14 showed that release into the mucus occurred soon after challenge infection, and correlated with a reduction in parasitic burden (Robinson et al. 2011). Additional it has been shown that LGAL-14 can bind directly to another parasite *Fasciola hepatica* suggesting it can inhibit infection.

The second galectin (LGALS-11) is specifically expressed and secreted during *H. contortus* infections in previously infected sheep that had developed resistance to the parasite (Dunphy et al. 2000). Immunohistochemistry revealed that LGALS-11 was secreted by epithelial cells lining the gastrointestinal tract, where it was localised to the nucleus and cytoplasm of cells. Analysis of the mucosal contents lining the gastrointestinal tract also revealed secretion of LGALS-11 into the mucus. An observation of increased mucus stickiness corresponding with the production of

66 LGALS-11 suggested that LGALS-11 might work by interacting with the mucus to impede *H.*
67 *contortus* motility (Robinson et al. 2011). Recent immunofluorescent staining techniques using a
68 recombinant form of galectin-11 have revealed binding to the fourth larval stage and adult *H.*
69 *contortus* that has resulted in impaired development. These studies suggest a more direct or
70 additional role for LGALS-11 during *H. contortus* infections.

71 It appears that both LGALS-11 and LGALS-14 mediate critical immune regulatory effects and/or
72 mediate direct parasite stage-specific killing (Haslam et al. 1998; Preston et al. 2015b). Although
73 the interactions of these host galectin-parasite glycoconjugates are likely to be critical for parasite
74 control, the parasite glycoconjugate molecules that they recognise are unknown. For the first
75 time, this study describes the ligands of sheep LGALS-11 and LGALS-14 in larval and adult
76 stages of *H. contortus*.

77 Materials and Methods

78 Preparation of L4 larvae and collection of adult parasites

79 *H. contortus* (Haecon-5 strain) was maintained in Professor Gasser's laboratory, Melbourne
80 Veterinary School, The University of Melbourne and was used in this study. Mature fourth stage
81 larvae (L4 stage) and adults of *H. contortus* were prepared using established protocols (Preston et
82 al. 2015a). Briefly, third-stage larvae (L3) were isolated from faeces from *H. contortus*-infected
83 sheep. The cuticle was removed from the L3s by using sodium hypochlorite, the exsheathed L3
84 (xL3) worms were washed three times with 0.9% (w/v) biological saline. Approximately 2000
85 xL3 / ml worms were resuspended in Dulbecco's modified Eagle Medium+GlutaMax (DMEM)
86 (Gibco-Invitrogen, USA) containing 10,000 IU/ml of penicillin and 10,000 µg/ml of
87 streptomycin (Gibco-Invitrogen, USA) and 0.5 % (v/v) fungicide (GE Healthcare, UK). Medium
88 containing xL3s was incubated at 37 °C with 10 % (v/v) CO_2 for 7 days. Fresh DMEM was
89 substituted at two-day intervals and larval development was examined each day. The xL3 and L4
90 stages were differentiated based on distinctive morphological characteristics (see Preston et al.,
91 2015a). Animal experimental procedures were approved by the Monash University Animal Ethics
92 Committee (Ethics # SOBSA/P/2009/44). Adults of *H. contortus* were collected from Merino
93 ewes (8-12 months old) which were experimentally infected with 10,000 L3s and the infected
94 animals were euthanised 52 days post infection by injection of pentobarbitone (Lethobarb®,
95 Virbac Pty Ltd, Australia). Approximately 5,000 adult worms of mixed sex were collected from

96 the abomasal content and washed five times with 0.9 % (v/v) biological saline (Baxter, Australia).
97 Immediately after washing, the worms were snap frozen in liquid nitrogen and stored at -80 °C
98 until further use.

99 Total larval protein lysate preparation

100 Lysates were prepared using radioimmunoprecipitation assay buffer (RIPA) as previously
101 described with minor modifications (Maduzia et al. 2011). Briefly, 500 mg of larval or adult *H.*
102 *contortus* were incubated with 100 mM β-D-galactose containing 0.9 % (v/v) biological saline
103 for 12 h to remove native galectins (bound to the adult parasite surface recovered from infected
104 sheep) and washed three times with normal saline. Larval or adult *H. contortus* were then
105 resuspended in 5 ml of ice-cold RIPA buffer [20 mM Tris-HCL pH 7.2, 100 mM NaCl, 1% (v/v)
106 Nonidet P-40, 0.1 % (w/v) sodium deoxycholate (DOC), 0.05 % (w/v) sodium dodecyl sulphate
107 (SDS), 1 % (v/v) Triton X-100, 10 mM TCEP (Tris (2-carboxyethyl) phosphine)] and lysed by
108 sonication (30 sec, 8 times with three min interval at 40 % amplitude). Cellular debris was
109 removed by centrifugation (15000 x *g* for 20 min) at 4 °C, and any particles in the supernatant
110 removed by filtering through a 0.22 µm filter. Lysates were dialysed using 3 kDa molecular
111 weight cut-off against binding buffer [(20 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.5 % (v/v)
112 Nonidet P-40, 0.1 % (w/v) DOC, 0.05 % (w/v) SDS, 1% (v/v) Triton X-100, 10 mM TCEP)].
113
114 SDS–PAGE

115 LGALS-11 and LGALS-14 affinity column

116 Recombinant LGALS-11 and LGALS-14 were expressed and purified as described previously
117 ((Sakthivel et al. 2015); Fig. 1). The recombinant protein (5 mg/ml) was buffer-exchanged into
118 HEPES buffer (10 mM HEPES-NaOH pH 7.5, 100 mM NaCl, 10 mM TCEP) and immobilised
119 by coupling to N-hydroxysuccinamide (NHS)-activated sepharose (GE Healthcare, UK)
120 following the manufacturer's protocol. Briefly, 4 ml of NHS-activated sepharose was washed
121 with 15 column-volumes of ice-cold 1 mM HCl. The washed Sepharose beads were equilibrated
122 with 20 ml of coupling buffer (10 mM HEPES-NaOH pH 7.5, 100 mM NaCl, 10 mM TCEP).
123 Following equilibration, LGALS-11 and LGALS-14 were added separately to the activated
124 Sepharose and allowed to couple for 5 h at 22 °C. Following the coupling reaction, the unused,
125 activated sites were blocked using 15 column-volumes of blocking buffer (100 mM Tris-HCl pH
126 8.0, 100 mM NaCl) for 3 h. Following blocking, the sepharose beads were washed alternatively
127 six times with 15 column-volumes of 100 mM Tris-HCL pH 8.0 and 100 mM sodium acetate pH

5.0 and 250 mM NaCl. The galectin affinity column was maintained in storage buffer (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM TCEP, NaAc 0.02 % (w/v)) until further use. A control resin was also prepared without any protein ligand.

Isolation of LGALS-11 and LGALS-14 parasite ligands

Immobilised LGALS-11, -14 or control slurry (1 ml) was loaded into individual columns. Larval and adult *H. contortus* lysates were diluted with 5 ml of binding buffer (20 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.5 % (v/v) Nonidet P-40, 0.1 % (w/v) DOC, 0.05 % (w/v) SDS, 1% (v/v) Triton X-100, 10 mM TCEP) and applied to the galectin affinity column and incubated for 16 h at 4°C. Thereafter, columns were washed three times with 15 ml of RIPA buffer, the captured protein fractions were eluted by incubating for 2 h with galactose elution buffer (250 mM β-D-Galactose 20 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM TCEP) and the resultant supernatant was subjected to LC-MS/MS analysis to identify the protein molecules present. The eluted protein products were analysed by 12% SDS-PAGE stained with nitrate. The unbound fractions, column wash and eluted proteins fractions were concentrated using sodium deoxycholate/trichloroacetic acid precipitation method to allow the visualisation of protein products as previously described (Arnold & Ulbrich-Hofmann 1999).

Mass spectrometric (ESI–LC–MS/MS) analysis of galectin binding proteins

Eluted protein samples were dissolved in digestion buffer (8 M urea, 50 mM NH_4HCO_3, 10 mM dithiothreitol) and incubated at 25 °C for 5 h. Following incubation, iodoacetamide (IAA) was added to final concentration of 55 mM to alkylate thiol groups and incubated for 35 min at 20 °C in the dark. The alkylated protein preparation was diluted with 1M urea in 25 mM ammonium bicarbonate (pH 8.5) and sequencing-grade trypsin (Promega) was added to a final concentration of 5 µM. The reaction was incubated for 16 h at 37 °C in the dark. The digests were acidified with 1% (v/v) trifluoroacetic acid (TFA) and the peptides desalted on poly(styrene-divinylbebzebe) copolymer (SDB) (Empore) StageTips 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 ID × 2 cm trapping column, Thermo-

156 Fisher Scientific) at 5 µl/min and washed for 6 min before switching the guard column, in line
157 with the analytical column (Vydac MS C_{18}, 3 µm, 300 Å and 75 µm ID × 25 cm). The separation
158 of peptides was performed at 300 nl/min using a non-linear ACN gradient of buffer A (0.1% (v/v)
159 formic acid, 2 % (v/v) ACN) and buffer B (0.1% (v/v) formic acid, 80 % (v/v) ACN), starting at
160 5% (v/v) buffer B to 55% for 120 min. Data were collected on an Orbitrap Elite (Thermo-Fisher
161 Scientific) in a Data-Dependent Acquisition mode using m/z 300–1500 as MS scan range, CID
162 MS/MS spectra and were collected for the 20 most intense ions. Dynamic exclusion parameters
163 were set as described previously (Nguyen et al. 2016). The Orbitrap Elite was operated in dual
164 analyser mode, with the Orbitrap analyser being used for MS and the linear trap being used for
165 MS/MS. Pull-down and LC-MS/MS analysis were performed three times on different days.

166 Database search and protein identification

167 The MS/MS spectra obtained from the Orbitrap analyser was used to search against the Swiss-
168 Prot *Haemonchus contortus* FASTA database (downloaded on 07.31.2016, 21,201 protein entries)
169 together with common contaminants were used for this analysis using the Mascot search engine
170 (Matrix Science Ltd., London, UK) as described previously (Perkins et al. 1999). Briefly,
171 carbamidomethylation of cysteines was set as a fixed modification, acetylation of protein N-
172 termini, methionine oxidation was included as variable modifications. Precursor mass tolerance
173 was 10 ppm, product ions were searched at 0.5 Da tolerances, minimum peptide length defined at
174 6, maximum peptide length 144, and Peptide spectral matches (PSM) were validated using
175 Percolator based on q-values at a 1% false discovery rate (FDR). Both peptide and protein
176 identifications were reported at a false discovery rate (FDR) of 1%. The mass spectrometry
177 proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner
178 repository with the data set identifier PXD008435 and 10.6019/PXD008435.

179 Protein-protein interaction analysis and visualisation

180 Normalized spectral abundance factor (NSAF) scores were calculated for the identified proteins
181 using the Scaffold software v4.7.2 (Searle 2010). Then proteins were subjected to the significance
182 analysis of interactome' (SAINT) (Choi et al. 2011) to identify *bona fide* protein-protein
183 interactions after removing all zero or missing rows. Proteins with a SAINT probability greater
184 than 0.9 were selected as high probability interactions. Finally, the resulting interaction network
185 was visualised using the Cytoscape v3.4.0 (Shannon et al. 2003).

186 **Analysis of glycosylation**

187 The N- and O-linked glycosylation pattern and the signal peptides of eluted proteins were
188 analysed following the instructions provided in the glycosylation analysis server. Briefly, N-
189 glycosylation and Signal peptide was analysed using NetNGlyc 1.0 server
190 (http://www.cbs.dtu.dk/services/NetNGlyc/). Whereas the O-glycosylation pattern was analysed
191 using NetOGlyc 4.0 Server (http://www.cbs.dtu.dk/services/NetOGlyc/). The results obtained
192 from the N and O glycosylation servers were provided as supplementary results.

193 Results and Discussion

194 **Identification of proteins of *H. contortus* that interacting with LGALS-11 and LGALS-14**
195 The overall experimental procedure to map interacting proteins of LGALS-11 and LGALS-14
196 from *H. contortus* is given in Fig. 1. Lysates of L4 and adult stages were assessed before loading
197 onto the columns containing the Sepharose immobilised LGALS-11 and LGALS-14 and are
198 shown in Fig. 2a. Multiple bands were observed, with both larval and adult lysates containing a
199 broad range of molecules of differing molecular weights. Following the application of L4 and
200 adult lysates to affinity columns, containing immobilised galectins, bound parasite molecules
201 were eluted with galactose (Fig. 2b & 2c). The eluted molecules from both affinity columns and
202 an control column were subjected to LC-MS/MS. Proteins that were identified in 2 of the 3
203 biological replicates were included for further analysis and the proteins that were bound to
204 control resin (S1) were removed from the analysis. Overall, 43 individual proteins were identified
205 and grouped based on their respective known or putative biological function(s) (Table 1). The
206 greatest number of proteins identified was in the adult stage of *H. contortus*; with 34 proteins
207 binding to LGALS-11 and 35 proteins binding to LGALS-14. Of those identified proteins, 26
208 proteins were found to bind to both LGALS-11 and LGALS-14 (Fig. 3; Table 2). In the L4 larval
209 stage, LGALS-11 and LGALS-14 could bind to 0 and 2 proteins respectively.

210 **Composition of LGALS-11 and LGALS-14 ligands**

211 Approximately 69% of proteins in *H. contortus* that bound specifically to LGALS-11 and/or
212 LGALS-14 were inferred to be involved in metabolic and regulatory processes (Table 1, Fig. 4).
213 Most of these proteins (~ 70 %) were likely involved in metabolic activities, such as energy
214 metabolism, transcription andtranslation. These proteins predominantly included regulatory

215 enzymes, such as peptidases, carboxyl transferases, aldo-keto reductases, deoxynucleoside
216 kinase, dehydrogenase, amidinotransferase and RNA polymerase. Another protein group (~9 %)
217 identified represented structural proteins, such as actin, myosin and collagen (Table 1, Fig. 4).
218 Other proteins identified had putative roles in molecular transport (e.g., lipid and amino acid
219 transport) or had no assigned function(s) (Fig. 4). *In silico* analysis revealed that approximately
220 65% of the proteins of the adult stage, that bound specifically to LGALS-11 and LGALS-14 had
221 one or more potential glycosylation site (Table 2). On the contrary, about 35 % of adult stages
222 specific proteins that bound to LGALS-11 and LGALS-14 were predicted as non-glycosylated.
223 Though animal lectins have a primary preference for glycoconjugates, it is believed that the
224 LGALS-11 and LGALS-14 might also display a glycan independent protein-protein interaction
225 activity similar to previously reported for galectin-1 and galectin-3 (Bawumia et al. 2003; Camby
226 et al. 2006; Menon et al. 2000; Paz 2001).

227 **Larval and adult ligands of LGALS-11 and LGALS-14**

228 More putative ligands (n = 43) were identified in the adult stage of *H. contortus* compared with
229 larval stages (n = 2) following galectin pull-down assays. Although the L4 stage is a histotropic
230 stage (in glands of the stomach) and would be expected to be in intimate contact with
231 inflammatory mediators, including galectins, it moults (with a change in antigenic profile) within
232 48-72 h into the immature adult (Meeusen et al. 2005). This would be expected to limit the
233 antigenic exposure of these parasite antigens to the host. Compared to the adult stage that is
234 relatively long-lived (6-8 weeks), allowing a sustained interaction of host molecules with parasite
235 antigens (Nikolaou & Gasser 2006; Veglia 1915). This interaction might be reflected in the
236 specific and localised binding of LGALS-11 in the larvae and the significant staining of LGALS-
237 11 on the surface of adult *H. contortus* (see Preston et al., 2015b). In addition, the L4 stage is
238 relatively small (750 – 850 μm long), whereas the adult stage is usually 10-30 mm long.

239 A protein-protein interaction network was drawn for LGALS-11 and LGALS-14 affinity purified
240 proteins specific to adult parasitic stage revealed that, LGALS-11 and LGALS-14 found to
241 interact 5 unique proteins individually. Whereas 9 proteins were found to interact with both
242 LGALS-11 and LGALS-14 (Fig. 5). Carboxyl transferase, Aldo keto reductase and myosin
243 displayed unique interaction with LGALS-11. Whereas Zinc metallopeptidase M13, Porin
244 domain containing protein, von Willebrand factor and mitochondrial solution substrate carrier

245 protein displayed an interaction network unique to LGALS-14. Peptidase S28, Alpha beta
246 hydrolase fold-1, Glutamate phenylalanine leucine valine dehydrogenase, Nematode cuticle
247 collagen, Lipid transport protein, Vitellinogen and von Willebrand factor domain were found to
248 interact both LGALS-11 and LGALS-14 (Fig. 5).

249 **Protease and phosphatase ligands**

250 A significant number of proteins (n = 19) with enzyme activity in adults were identified, and
251 similar proteins have been described in other 'omic studies, suggesting that many of these
252 enzymes of the protease family are conserved and evolutionarily related in nematodes (Campbell
253 et al. 2011; Ghedin et al. 2007; Schwarz et al. 2013). A notable protease identified in the adult
254 stage, is zinc metallopeptidase (M13 protease or neprilysin). Zinc metallopeptidases have been
255 reported as the major protein fraction of host protective glycoprotein complex H-gal-GP
256 (*Haemonchus* galactose containing glycoprotein). Several studies isolated zinc metallopeptidases
257 from crude extracts of *H. contortus* using lectins that have a binding preference to β-D-galactose
258 and, following vaccination of sheep, led to reduced worm burdens following challenge infection
259 (Dicker et al. 2014; Newlands GFJ 2006; Smith et al. 1999; Smith et al. 2000).

260 **Blood ligands**

261 A number of parasite molecules were identified that interact with host galectins and are
262 potentially involved in manipulating the host blood function in the adult stage but not larvae of
263 *H. contortus*. That the adult stage of this nematode is primary a blood feeder may explain the lack
264 of such molecules identified in the larvae. Blood feeding parasites are known to use several
265 mechanisms to suppress platelet aggregation, allowing prolonged blood feeding by retarding
266 blood clotting (Liu & Weller 1992). The von-Willebrant factor (VWF) domain is a well-known
267 protein domain reported in integrin and other extracellular proteins (Whittaker & Hynes 2002).
268 The binding of a C-type lectin (CLEC4M), with VWF has previously been shown to enhance the
269 internalisation of VWF by the host cells and alter plasma levels of VWF (Rydz et al. 2013). In
270 previous reports, proteins containing the VWF domain are localised in nematode intestine and
271 suggested to play critical roles in cell adhesion and platelet aggregation (Wohner et al. 2012). A
272 multimeric glycoprotein containing VWF domain was identified previously in adult *H. contortus*
273 that can suppress platelet aggregation (Crab et al. 2002). In this study, a protein containing a
274 VWF domain was eluted from the LGAL14 column, which might suggest that this host galectin

275 plays a role in potential modulating the ability of the parasite to suppress blood clotting. This
276 protein was not detected in larvae by both LGALS-11 and LGALS-14. However, the functional
277 significance of VWF in parasitised animals remains unknown, warranting further study.

278 **Specific sperm-coating protein (SCP)**

279 The stage-specific sperm-coating protein (SCP) identified by host galectins in this study are
280 common to many nematode species (Cantacessi & Gasser 2012) and are suggested to play critical
281 roles in infection and immunomodulatory events such as neutrophil inhibition (Cantacessi et al.
282 2012; Gadahi et al. 2016; Hewitson et al. 2009). Transcriptomic studies of *H. contortus* have
283 identified that 54 genes containing one or more SCP-like domains are upregulated in the blood-
284 feeding adult, suggesting that SCP proteins have active and stage-specific involvement at the
285 onset of blood feeding (Wang & Kim 2003). Similar SCP domain containing proteins (Hc24 and
286 Hc40) were reported in excretory/secretory proteins of *H. contortus (Yatsuda et al. 2003)*.
287 Although there is some information for SCP domain-containing proteins in *C. elegans* (O'Rourke
288 et al. 2006; Wang & Kim 2003), their biological functions in *H. contortus* needs experimental
289 investigation.

290 Conclusion

291 Recently, host galectins have been hypothesised to interact with molecules to modulate host-
292 pathogen interactions in ruminants (Hoorens et al. 2011; Kemp et al. 2009; Preston et al. 2015b).
293 The finding that LGAL-14 is concentrated within eosinophils (an immune cell considered a major
294 mediator of parasite killing, including of *H. contortus*) suggested the possibility of a direct role
295 for ruminant galectins in mediating parasite-killing (Meeusen & Balic 2000; Robinson et al.
296 2011). The subsequent demonstration of direct binding of LGAL-11 to *H. contortus* and their
297 ability to inhibit larval development and growth *in vitro* has confirmed the roles of galectins and
298 ability to directly kill relatively large multicellular pathogens (Preston et al. 2015b).

299 The parasite surface is the key contact with the host and is often considered important source of
300 potential vaccine molecules. Correspondingly, 45% of the glycoproteins that the two galectins
301 bound were membrane proteins of the adult stage of *H. contortus,* and included vitelline, myosin
302 and M13 protein (neprilysin); these proteins have been previously assessed as vaccine candidates
303 (Knox 2011; Strube et al. 2015; Tellam et al. 2002). This evidence would indicate that other

304 putative glycoproteins identified here by these ruminant galectins might facilitate the
305 identification of new intervention targets and, thus, warrant further investigation. In conclusion,
306 the analysis of parasite proteins recognised by galectins that are involved in resistance to
307 parasites (Guo et al. 2016; Preston et al. 2015a; Preston et al. 2015b), has identified several
308 interesting stage-specific proteins. Exploring the possible biological roles and potential
309 anthelminthic activities of these proteins has significant potential to advance our understanding of
310 the host-parasite interplay and inform future parasite control strategies.

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315 # References

316 Ackerman SJ, Liu L, Kwatia MA, Savage MP, Leonidas DD, Swaminathan GJ, and Acharya KR.
317 2002. Charcot-Leyden Crystal Protein (Galectin-10) Is Not a Dual Function Galectin with
318 Lysophospholipase Activity but Binds a Lysophospholipase Inhibitor in a Novel
319 Structural Fashion. *Journal of Biological Chemistry* 277:14859-14868.

320 Arnold U, and Ulbrich-Hofmann R. 1999. Quantitative Protein Precipitation from Guanidine
321 Hydrochloride-Containing Solutions by Sodium Deoxycholate/Trichloroacetic Acid.
322 *Analytical Biochemistry* 271:197-199. https://doi.org/10.1006/abio.1999.4149

323 Balic A, Cunningham CP, and Meeusen ENT. 2006. Eosinophil interactions with Haemonchus
324 contortus larvae in the ovine gastrointestinal tract. *Parasite Immunology* 28:107-115.
325 10.1111/j.1365-3024.2006.00816.x

326 Bawumia S, Barboni Eminia AM, Menon Rajesh P, and Colin Hughes R. 2003. Specificity of
327 interactions of galectin-3 with Chrp, a cysteine- and histidine-rich cytoplasmic protein.
328 *Biochimie* 85:189-194. https://doi.org/10.1016/S0300-9084(03)00007-5

329 Camby I, Le Mercier M, Lefranc F, and Kiss R. 2006. Galectin-1: a small protein with major
330 functions. *Glycobiology* 16:137R-157R. 10.1093/glycob/cwl025

331 Campbell BE, Hofmann A, McCluskey A, and Gasser RB. 2011. Serine/threonine phosphatases
332 in socioeconomically important parasitic nematodes—Prospects as novel drug targets?
333 *Biotechnology Advances* 29:28-39. http://dx.doi.org/10.1016/j.biotechadv.2010.08.008

334 Cantacessi C, and Gasser RB. 2012. SCP/TAPS proteins in helminths – Where to from now?
335 *Molecular and Cellular Probes* 26:54-59. https://doi.org/10.1016/j.mcp.2011.10.001

336 Cantacessi C, Hofmann A, Young ND, Broder U, Hall RS, Loukas A, and Gasser RB. 2012.
337 Insights into SCP/TAPS proteins of liver flukes based on large-scale bioinformatic
338 analyses of sequence datasets. *PLOS ONE* 7:e31164. 10.1371/journal.pone.0031164

339 Choi H, Larsen B, Lin Z-Y, Breitkreutz A, Mellacheruvu D, Fermin D, Qin ZS, Tyers M, Gingras
340 A-C, and Nesvizhskii AI. 2011. SAINT: Probabilistic Scoring of Affinity Purification -
341 Mass Spectrometry Data. *Nature methods* 8:70-73. 10.1038/nmeth.1541

342 Crab A, Noppe W, Pelicaen C, Hoorelbeke KV, and Deckmyn H. 2002. The parasitic
343 hematophagous worm *Haemonchus contortus* inhibits human platelet aggregation and
344 adhesion: partial purification of a platelet inhibitor. *Thrombosis and Haemostasis* 87:899-
345 904.

346 Dicker AJ, Inglis NF, Manson EDT, Subhadra S, Illangopathy M, Muthusamy R, and Knox DP.
347 2014. Proteomic analysis of *Mecistocirrus digitatus* and *Haemonchus contortus* intestinal
348 protein extracts and subsequent efficacy testing in a vaccine trial. *PLOS Neglected*
349 *Tropical Diseases* 8:e2909. 10.1371/journal.pntd.0002909

350 Dunphy JL, Balic A, Barcham GJ, Horvath AJ, Nash AD, and Meeusen ENT. 2000. Isolation and
351 characterization of a novel inducible mammalian galectin. *Journal of Biological*
352 *Chemistry* 275:32106-32113. 10.1074/jbc.M003739200

353 Dunphy JL, Barcham GJ, Bischof RJ, Young AR, Nash A, and Meeusen ENT. 2002. Isolation and
354 characterization of a novel eosinophil-specific galectin released into the lungs in response
355 to allergen challenge. *Journal of Biological Chemistry* 277:14916-14924.

356 Gadahi JA, Wang S, Bo G, Ehsan M, Yan R, Song X, Xu L, and Li X. 2016. Proteomic analysis
357 of the excretory and secretory proteins of *Haemonchus contortus* (HcESP) binding to goat
358 PBMCs *in vivo* revealed stage-specific binding profiles. *PLOS ONE* 11:e0159796.
359 10.1371/journal.pone.0159796

360 Ghedin E, Wang S, Spiro D, Caler E, Zhao Q, Crabtree J, Allen JE, Delcher AL, Guiliano DB,
361 and et al. 2007. Draft Genome of the Filarial Nematode Parasite Brugia malayi. *Science*
362 317:1756.

363 Guo Z, Gonzalez JF, Hernandez JN, McNeilly TN, Corripio-Miyar Y, Frew D, Morrison T, Yu P,
364 and Li RW. 2016. Possible mechanisms of host resistance to Haemonchus contortus
365 infection in sheep breeds native to the Canary Islands. *Sci Rep* 6:26200.
366 10.1038/srep26200

367 Haslam SM, Coles GC, Reason AJ, Morris HR, and Dell A. 1998. The novel core fucosylation of
368 *Haemonchus contortus* N-glycans is stage specific. *Molecular and Biochemical*
369 *Parasitology* 93:143-147. http://dx.doi.org/10.1016/S0166-6851(98)00020-6

370 Hewitson JP, Grainger JR, and Maizels RM. 2009. Helminth immunoregulation: The role of
371 parasite secreted proteins in modulating host immunity. *Molecular and Biochemical*
372 *Parasitology* 167:1-11. http://dx.doi.org/10.1016/j.molbiopara.2009.04.008

373 Hoorens P, Rinaldi M, Mihi B, Dreesen L, Grit G, Meeusen E, Li RW, and Geldhof P. 2011.
374 Galectin-11 induction in the gastrointestinal tract of cattle following nematode and
375 protozoan infections. *Parasite Immunology* 33:669-678. 10.1111/j.1365-
376 3024.2011.01336.x

377 Kemp JM, Robinson NA, Meeusen ENT, and Piedrafita DM. 2009. The relationship between the
378 rapid rejection of *Haemonchus contortus* larvae with cells and mediators in abomasal
379 tissues in immune sheep. *International Journal for Parasitology* 39:1589-1594.
380 http://dx.doi.org/10.1016/j.ijpara.2009.05.015

381 Knox D. 2011. Proteases in blood-feeding nematodes and their potential as vaccine candidates.
382 *Adv Exp Med Biol* 712.

383 Liu LX, and Weller PF. 1992. Intravascular filarial parasites inhibit platelet aggregation. Role of
384 parasite-derived prostanoids. *Journal of Clinical Investigation* 89:1113-1120.

385 Maduzia LL, Yu E, and Zhang Y. 2011. Caenorhabditis elegans Galectins LEC-6 and LEC-10
386 Interact with Similar Glycoconjugates in the Intestine. *Journal of Biological Chemistry*
387 286:4371-4381.

388 Mavrot F, Hertzberg H, and Torgerson P. 2015. Effect of gastro-intestinal nematode infection on
389 sheep performance: a systematic review and meta-analysis. *Parasites & Vectors* 8:557.
390 10.1186/s13071-015-1164-z

391 McLeod RS. 1995. Cost of major parasites to the Australian livestock industries. *Int J Parasitol*
392 25. 10.1016/0020-7519(95)00071-9

393 Meeusen ENT, and Balic A. 2000. Do Eosinophils have a Role in the Killing of Helminth
394 Parasites? *Parasitology Today* 16:95-101. 10.1016/S0169-4758(99)01607-5

395 Meeusen ENT, Balic A, and Bowles V. 2005. Cells, cytokines and other molecules associated
396 with rejection of gastrointestinal nematode parasites. *Veterinary Immunology and*
397 *Immunopathology* 108:121-125. http://dx.doi.org/10.1016/j.vetimm.2005.07.002

398 Menon RP, Strom M, and Hughes RC. 2000. Interaction of a novel cysteine and histidine-rich
399 cytoplasmic protein with galectin-3 in a carbohydrate-independent manner. *FEBS Letters*
400 470:227-231. 10.1016/S0014-5793(00)01310-7

401 Newlands GFJ SP, Nisbet AJ, Redmond DL, Smith SK, Petitit D, Smith WD. 2006. Molecular
402 characterization of a family of metalloendopeptidases from the intestinal brush border of
403 Haemonchus contortus. *Parasitology* 133:357–368.

404 Nguyen VA, Carey LM, Giummarra L, Faou P, Cooke I, Howells DW, Tse T, Macaulay SL, Ma
405 H, Davis SM, Donnan GA, and Crewther SG. 2016. A Pathway Proteomic Profile of
406 Ischemic Stroke Survivors Reveals Innate Immune Dysfunction in Association with Mild
407 Symptoms of Depression – A Pilot Study. *Frontiers in Neurology* 7:85.
408 10.3389/fneur.2016.00085

409 Nikolaou S, and Gasser RB. 2006. Prospects for exploring molecular developmental processes in
410 *Haemonchus contortus*. *International Journal for Parasitology* 36:859-868.
411 http://dx.doi.org/10.1016/j.ijpara.2006.04.007

412 Nisbet AJ, Meeusen EN, González JF, and Piedrafita DM. 2016. Chapter Eight - Immunity to
413 Haemonchus contortus and Vaccine Development. In: Robin BG, and Georg Von S-H,
414 eds. *Advances in Parasitology*: Academic Press, 353-396.

415 O'Rourke D, Baban D, Demidova M, Mott R, and Hodgkin J. 2006. Genomic clusters, putative
416 pathogen recognition molecules, and antimicrobial genes are induced by infection of C.
417 elegans with M. nematophilum. *Genome Research* 16:1005-1016. 10.1101/gr.50823006

418 Paz AH, R.; Elad-Sfadia, G.; Ballan, E.; Kloog, Y. 2001. Galectin-1 binds oncogenic H-Ras to
419 mediate Rasmembrane anchorage and cell transformation. *Oncogene* 20:7486-7493.

420 Perkins DN, Pappin DJC, Creasy DM, and Cottrell JS. 1999. Probability-based protein
421 identification by searching sequence databases using mass spectrometry data.
422 *ELECTROPHORESIS* 20:3551-3567.

423 Preston S, Dunphy J, Beddoe T, Meeusen E, and Young A. 2015a. Evaluation of the role of
424 galectins in parasite immunity. In: Stowell SR, and Cummings RD, eds. *Galectins:*
425 *Methods and Protocols*. New York, NY: Springer New York, 371-395.

426 Preston SJM, Beddoe T, Walkden-Brown S, Meeusen E, and Piedrafita D. 2015b. Galectin-11: A
427 novel host mediator targeting specific stages of the gastrointestinal nematode parasite,
428 Haemonchus contortus. *International Journal for Parasitology* 45:791-796.
429 http://dx.doi.org/10.1016/j.ijpara.2015.06.003

430 Rappsilber J, Mann M, and Ishihama Y. 2007. Protocol for micro-purification, enrichment, pre-
431 fractionation and storage of peptides for proteomics using stage tips. *Nat Protocols*
432 2:1896-1906.

433 Robinson N, Pleasance J, Piedrafita D, and Meeusen EN. 2011. The kinetics of local cytokine and
434 galectin expression after challenge infection with the gastrointestinal nematode,
435 Haemonchus contortus. *International Journal for Parasitology* 41:487-493.
436 10.1016/j.ijpara.2010.11.006

437 Roeber F, Jex AR, and Gasser RB. 2013. Impact of gastrointestinal parasitic nematodes of sheep,
438 and the role of advanced molecular tools for exploring epidemiology and drug resistance -
439 an Australian perspective. *Parasites & Vectors* 6:153-153. 10.1186/1756-3305-6-153

440 Rydz N, Swystun LL, Notley C, Paterson AD, Riches JJ, Sponagle K, Boonyawat B,
441 Montgomery RR, James PD, and Lillicrap D. 2013. The C-type lectin receptor CLEC4M
442 binds, internalizes, and clears von Willebrand factor and contributes to the variation in
443 plasma von Willebrand factor levels. *Blood* 121:5228-5237. 10.1182/blood-2012-10-
444 457507

445 Sakthivel D, Littler D, Shahine A, Troy S, Johnson M, Rossjohn J, Piedrafita D, and Beddoe T.
446 2015. Cloning, expression, purification and crystallographic studies of galectin-11 from
447 domestic sheep (*Ovis aries*). *Acta Crystallographica Section F* 71:993-997.
448 doi:10.1107/S2053230X15010195

449 Schwarz EM, Korhonen PK, Campbell BE, Young ND, Jex AR, and Jabbar A. 2013. The genome
450 and developmental transcriptome of the strongylid nematode Haemonchus contortus.
451 *Genome Biol* 14. 10.1186/gb-2013-14-8-r89

452 Searle BC. 2010. Scaffold: A bioinformatic tool for validating MS/MS-based proteomic studies.
453 *PROTEOMICS* 10:1265-1269. 10.1002/pmic.200900437

454 Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, and
455 Ideker T. 2003. Cytoscape: A Software Environment for Integrated Models of
456 Biomolecular Interaction Networks. *Genome Research* 13:2498-2504.
457 10.1101/gr.1239303

458 Smith SK, Pettit D, Newlands GF, Redmond DL, Skuce PJ, Knox DP, and Smith WD. 1999.
459 Further immunization and biochemical studies with a protective antigen complex from the
460 microvillar membrane of the intestine of Haemonchus contortus. *Parasite Immunol*
461 21:187-199.

462 Smith WD, Smith SK, Pettit D, Newlands GF, and Skuce PJ. 2000. Relative protective properties
463 of three membrane glycoprotein fractions from Haemonchus contortus. *Parasite Immunol*
464 22:63-71.

465 Strube C, Haake C, Sager H, Schorderet Weber S, Kaminsky R, Buschbaum S, Joekel D, Schicht
466 S, Kremmer E, Korrell J, Schnieder T, and von Samson-Himmelstjerna G. 2015.
467 Vaccination with recombinant paramyosin against the bovine lungworm Dictyocaulus
468 viviparus considerably reduces worm burden and larvae shedding. *Parasites & Vectors*
469 8:119. 10.1186/s13071-015-0733-5

470 Tellam RL, Kemp D, Riding G, Briscoe S, Smith D, Sharp P, Irving D, and Willadsen P. 2002.
471 Reduced oviposition of Boophilus microplus feeding on sheep vaccinated with vitellin.
472 *Veterinary Parasitology* 103:141-156. http://dx.doi.org/10.1016/S0304-4017(01)00573-8

473 Veglia F. 1915. The anatomy and life history of the *Haemonchus contortus* (Rud). *Report on*
474 *Veterinary Research Department of Agriculture, Union of South Africa* 3:349-500.

475 Wang J, and Kim SK. 2003. Global analysis of dauer gene expression in Caenorhabditis elegans.
476 *Development* 130:1621.

477 Whittaker CA, and Hynes RO. 2002. Distribution and evolution of von willebrand/integrin A
478 domains: widely dispersed domains with roles in cell adhesion and elsewhere. *Molecular*
479 *Biology of the Cell* 13:3369-3387.

PeerJ Preprints NOT PEER-REVIEWED

480 Wohner N, Kovács A, Machovich R, and Kolev K. 2012. Modulation of the von Willebrand
481 factor-dependent platelet adhesion through alternative proteolytic pathways. *Thrombosis*
482 *Research* 129:e41-e46. 10.1016/j.thromres.2011.11.021

483 Yatsuda AP, Krijgsveld J, Cornelissen AW, Heck AJ, and de Vries E. 2003. Comprehensive
484 analysis of the secreted proteins of the parasite Haemonchus contortus reveals extensive
485 sequence variation and differential immune recognition. *J Biol Chem* 278:16941-16951.
486 10.1074/jbc.M212453200

487 Young AR, Barcham GJ, Kemp JM, Dunphy JL, Nash A, and Meeusen EN. 2009. Functional
488 characterization of an eosinophil-specific galectin, ovine galectin-14. *Glycoconjugate*
489 *Journal* 26:423-432. 10.1007/s10719-008-9190-0

Table 1 (on next page)

Identification by mass spectroscopy of larval and adult *Haemonchus contortus* proteins eluted from LGALS-11 and LGALS-14 columns

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Accession code	Gene description	CV	PSMs	UP	Mascot Score	Groups				Log-odds
						1	2	3	4	
Metabolic process										
W6NE18	Peptidase S28 GN=HCOI_01497800	45.21	634	20	7058	No	Yes	Yes	Yes	145.7
W6NFG0	Alpha beta hydrolase fold-1 GN=HCOI_00457700	58.65	504	16	6681	No	Yes	Yes	Yes	128.68
W6NFT9	Peptidase S28 GN=HCOI_01562400	23.27	222	18	3151	No	Yes	No	Yes	11.26
W6NJ96	Carboxyl transferase GN=HCOI_00766200	23.39	123	10	1146	No	Yes	No	Yes	16.01
W6NLA8	Glutamate phenylalanine leucine valine dehydrogenase	42.50	102	10	957	No	Yes	No	Yes	20.61
W6NG90	Peptidase S28 GN=HCOI_01624000	4.88	101	3	1218	No	Yes	No	Yes	29.6
W6NC58	Aldo keto reductase GN=HCOI_00043700	39.64	83	10	789	No	Yes	No	Yes	-0.18
W6NAV8	Aldo keto reductase GN=HCOI_00043500	27.22	46	6	477	No	Yes	No	Yes	-0.18
W6NU27	Carboxyl transferase GN=HCOI_00766300	25.83	67	6	909	No	Yes	No	Yes	4.35
W6NKM1	Succinate dehydrogenase iron-sulfur subunit, GN=HCOI_01735500	32.61	64	8	521	No	Yes	No	Yes	34.83
W6NF70	Deoxynucleoside kinase	35.42	42	6	242	No	No	No	Yes	-0.18
U6NNG6	Ribosomal protein L7 L12 GN=HCOI_00340500	11.41	30	1	783	No	Yes	No	Yes	-0.18
W6NA79	Zinc metallopeptidase M13 GN=HCOI_01030800	37.90	28	3	330	No	No	No	Yes	4.35
W6ND82	von Willebrand factor GN=HCOI_01354500	13.82	26	3	371	No	No	No	Yes	16.01

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W6NMI7	Proteinase inhibitor I33 GN=HCOI_02015200	18.58	22	5	162	No	No	No	Yes	-0.18
W6NKG5	Ribosomal protein L15 GN=HCOI_01717500	5.88	4	1	26	No	No	No	Yes	5.67
W6NEW9	Amidinotransferase GN=HCOI_01556200	23.35	24	4	109	No	Yes	No	No	-0.18
W6NI22	Adenylosuccinate lyase GN=HCOI_00436500	6.41	14	3	141	No	Yes	No	No	12.23
W6NF84	Short-chain dehydrogenase reductase GN=HCOI_01467500	3.07	11	1	137	No	Yes	No	Yes	1.71
W6ND43	Acetyltransferase component of pyruvate dehydrogenase complex GN=HCOI_00576100	1.22	8	1	51	No	Yes	No	No	1.68
	Regulation of biological processes									
W6NC73	ATPase GN=HCOI_02138200	22.64	23	2	418	No	Yes	No	Yes	-0.18
W6NJ12	Filament domain containing protein GN=HCOI_02013700	14.09	20	3	291	No	Yes	No	Yes	-0.18
W6NM02	Fumarate lyase GN=HCOI_01914600	4.73	8	1	62	No	Yes	No	No	4.61
W6NGK5	CRE-DHS-15 protein GN=HCOI_00341400	29.31	6	1	210	No	No	No	Yes	-0.18
W6NI80	Acyl-CoA-binding protein GN=HCOI_01539100	20.69	6	1	205	No	Yes	No	Yes	6.69
W6NCC1	NIPSNAP GN=HCOI_01963300	7.63	6	1	75	No	Yes	No	No	-0.18
W6NWY9	Porin domain containing protein GN=HCOI_01573900	43.97	82	9	1149	No	Yes	No	Yes	4.35
W6NAW4	FG-GAP and Integrin alpha-2/Integrin alpha chain GN=HCOI_01903100	5.90	33	5	340	No	Yes	No	Yes	-0.18
W6NAL4	Heat shock protein 70 GN=HCOI_00589700	7.24	9	1	82	No	Yes	No	No	7.23

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	Transport									
W6NVQ1	Lipid transport protein and Vitellinogen and von Willebrand factor domain GN=HCOI_01683400	23.18	209	24	1465	No	Yes	No	Yes	9.62
W6N9I2	Lipid transport protein GN=HCOI_00072100	35.97	139	13	1313	No	Yes	No	Yes	51.51
W6NQZ5	Mitochondrial substrate solute carrier GN=HCOI_01092000	23.10	53	8	334	No	No	No	Yes	6.2
	Cytoskeleton									
W6NAH7	Nematode cuticle collagen and Collagen triple helix repeat	5.60	85	2	1399	No	Yes	No	Yes	4.35
W6NHH0	Annexin GN=HCOI_01003500	14.01	22	3	135	No	Yes	No	No	-0.18
W6NE41	Myosin tail GN=HCOI_01216000	3.99	17	3	59	No	Yes	No	No	13.42
W6NF56	Myosin tail GN=HCOI_01461300	34.48	51	8	675	No	Yes	No	Yes	6.2
	Host-parasite interaction									
W6NGA7	SCP extracellular domain GN=HCOI_01577700	14.95	4	2	59	No	Yes	No	Yes	-0.18
	Unknown									
W6NAN9	CBN-MLC-3 protein GN=HCOI_01274700	49.67	63	7	465	No	Yes	No	Yes	2.32
W6NX42	Uncharacterized protein GN=HCOI_01051700	25.56	38	3	607	No	No	No	Yes	19.09
W6NFI4	Protein C15F1.2 GN=HCOI_01126300	34.87	20	4	97	No	Yes	No	Yes	-0.18
W6NB42	Protein C23H5.8, isoform-c GN=HCOI_00648100	14.43	16	2	170	No	Yes	No	Yes	10.80
W6NPK3	Uncharacterized protein GN=HCOI_00260500	5.20	10	1	45	No	No	No	Yes	-0.18
W6NUX4	Uncharacterized protein GN=HCOI_01608400	4.04	12	1	43	No	Yes	No	Yes	4.04

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Abbreviations:
GN = Gene name; CV = Coverage; PSMs = Peptide spectrum matches; UP = Unique peptides;
Log-odds 0 = 50% chance, Positive values = More than 50%, Negative values = Less than 50%
Groups:
1 = LGALS-11 bound protein from L4 larval stage of *H. contortus*
2 = LGALS-11 bound protein from adult stage of *H. contortus*
3 = LGALS-14 bound protein from L4 larval stage of *H. contortus*
4 = LGALS-14 bound protein from adult stage of *H. contortus*

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Table 2(on next page)

Host galectins LGALS-11 and LGALS-14 ligands common to adult stages of *H. contortus*

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Accession	Gene Description	Signal peptide	Potential Glycosylation
W6NE18	Peptidase S28 domain containing protein GN=HCOI_01497800	No	Yes
W6NFG0	Alpha beta hydrolase fold-1 GN=HCOI_00457700	Yes	Yes
W6NFT9	Peptidase S28 domain containing protein GN=HCOI_01562400	Yes	Yes
W6NVQ1	Lipid transport protein and Vitellinogen and von Willebrand factor domain containing protein GN=HCOI_01683400	No	No
W6N9I2	Lipid transport protein GN=HCOI_00072100 PE=4 SV=1	Yes	No
W6NJ96	Carboxyl transferase GN=HCOI_00766200	No	No
W6NLA8	Glutamate phenylalanine leucine valine dehydrogenase	No	Yes
W6NG90	Peptidase S28 domain containing protein GN=HCOI_01624000	Yes	Yes
W6NAH7	Nematode cuticle collagen	No	No
W6NC58	Aldo keto reductase GN=HCOI_00043700	No	Yes
W6NWY9	Porin domain containing protein GN=HCOI_01573900	No	Yes
W6NU27	Carboxyl transferase GN=HCOI_00766300	No	Yes
W6NKM1	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial GN=HCOI_01735500	No	Yes
W6NAN9	CBN-MLC-3 protein GN=HCOI_01274700	No	Yes
W6NF56	Myosin GN=HCOI_01461300	No	No
W6NAV8	Aldo keto reductase GN=HCOI_00043500	No	Yes
W6NAW4	FG-GAP and Integrin alpha-2 and Integrin alpha	Yes	Yes
U6NNG6	Ribosomal protein L7 L12 GN=HCOI_00340500	No	Yes
W6NEW9	Amidinotransferase	No	No
W6NC73	ATPase domain containing protein	No	Yes
W6NJT2	Filament domain containing protein GN=HCOI_02013700	No	No

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Abbreviations:
GN = Gene name

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Figure 1 (on next page)

Schematic flow of pull-down experiment to identify the interactome.

Lysates of *Haemonchus contortus* (larval or adult worms) containing glycoproteins were isolated using immobilised recombinant LGALS-11 and LGALS-14 columns and eluted using a high concentration of β-D-galactose. The glycoproteins of larval and adult stages that interact with host galectins were analysed by LC-MS/MS. The spectra obtained from the LC-MS/MS were analysed using the Mascot (Perkins et al. 1999) and the NCBI protein database.

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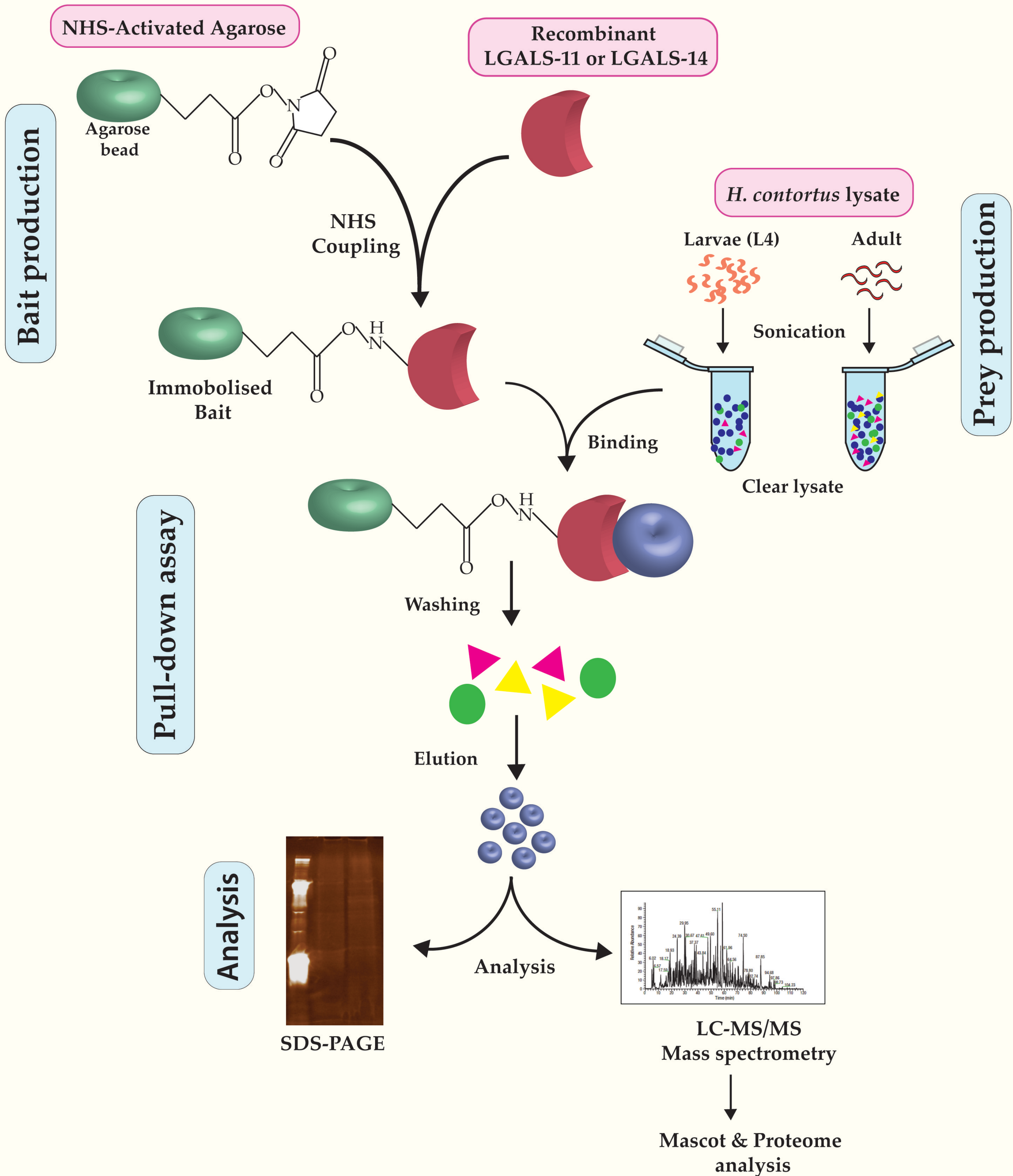
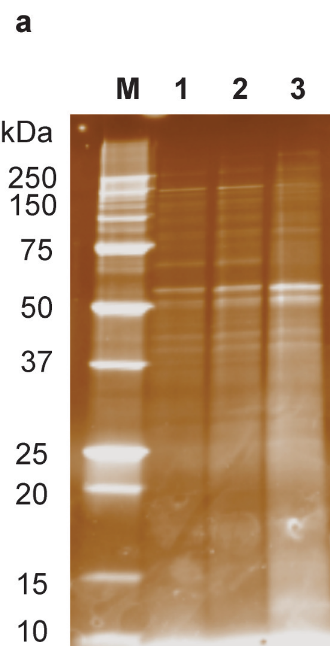


Figure 2(on next page)

SDS-PAGE analysis of galectin bound proteins.

(A) Protein profiling of larval and adult stages of *Haemonchus contortus*. (M) Molecular weight markers; (Lane 1 & 2) lysates prepared from L4 stage; (Lane 3) lysates prepared from adult stage**. (B) Protein profile of adult stage parasite bound to LGALS-11 and LGALS-14 and (C) larval stage parasite bound to LGALS-11 and LGALS-14.** M) Molecular weight markers; (Lane 1) Total parasite lysate, (Lane 2 & 3) Unbound protein fractions of LGALS-11 and LGALS-14 column, (Lane 4 & 5) Column wash of LGALS-11 and LGALS-14 column and (Lane 6 & 7) eluted protein of LGALS-11 and LGALS-14 column.

a



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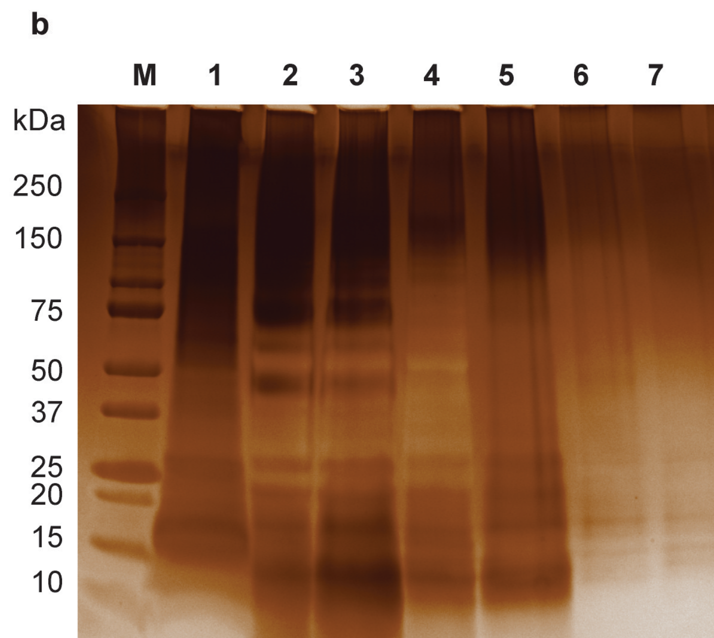

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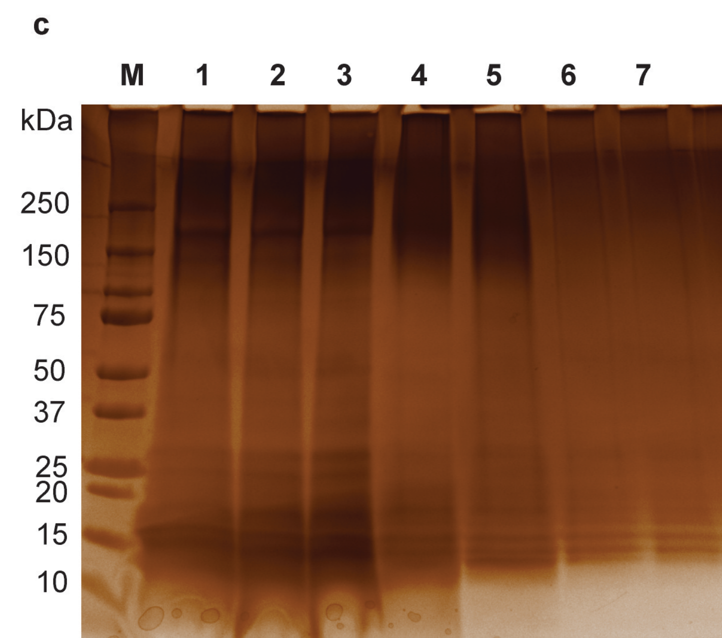

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Figure 3(on next page)

Venn diagram of parasite proteins bound by host galectins.

Venn diagram showing the distribution of proteins of the larval (A) and adult (B) stages of *Haemonchus contortus*. In larval and adult stages, 0 and 26 proteins were bound by both the galectins respectively.

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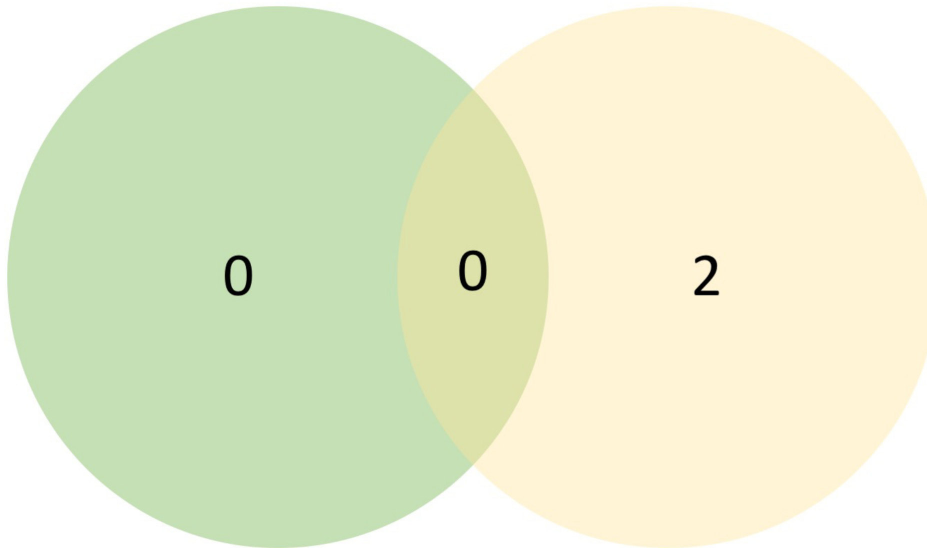

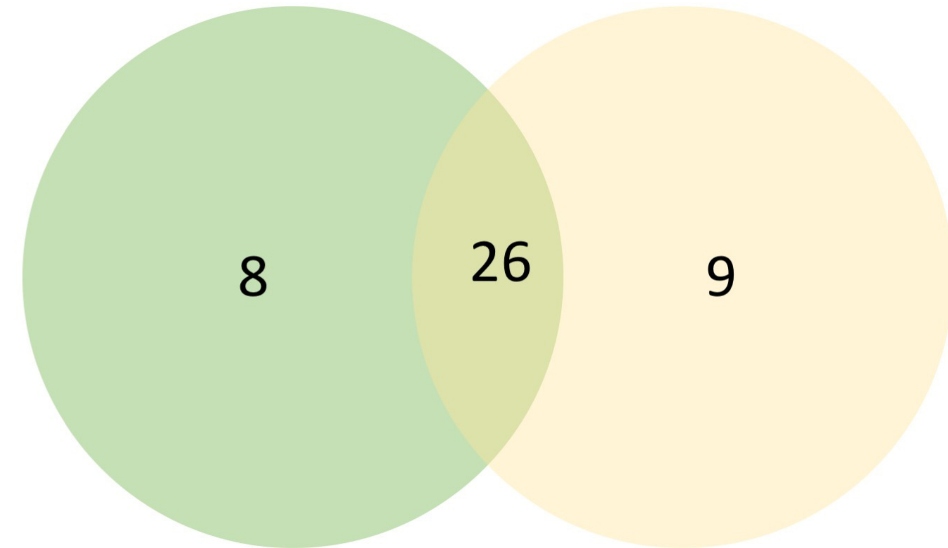

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Figure 4 (on next page)

Categorisation of proteins in the adult stage of *Haemonchus contortus* that interacted with host galectins.

The profiles were categorised based on biological process of LGALS-11-bound proteins (A) and LGALS-14 bound-proteins (B) and cellular location of LGALS-11-bound proteins (C) and LGALS-14-bound proteins (D).

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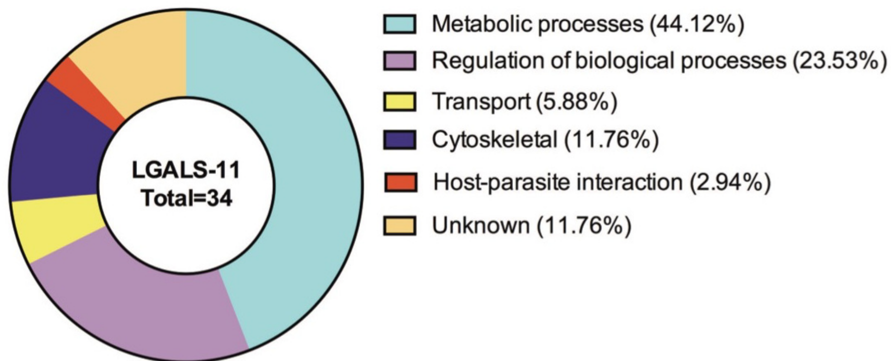
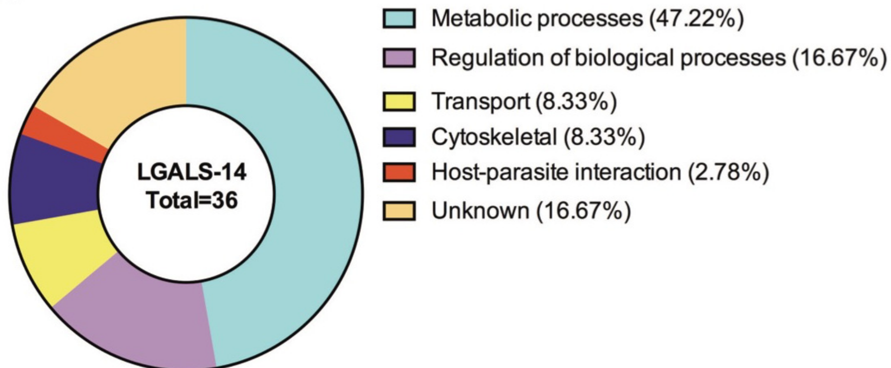
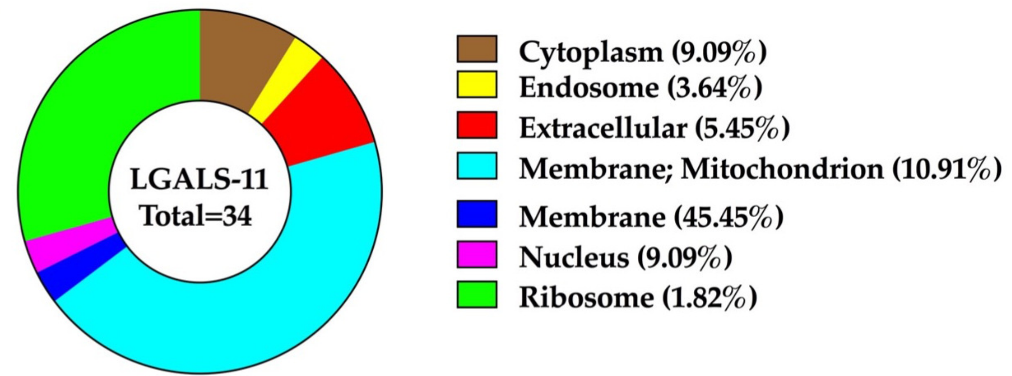
Biological Process





Cellular component



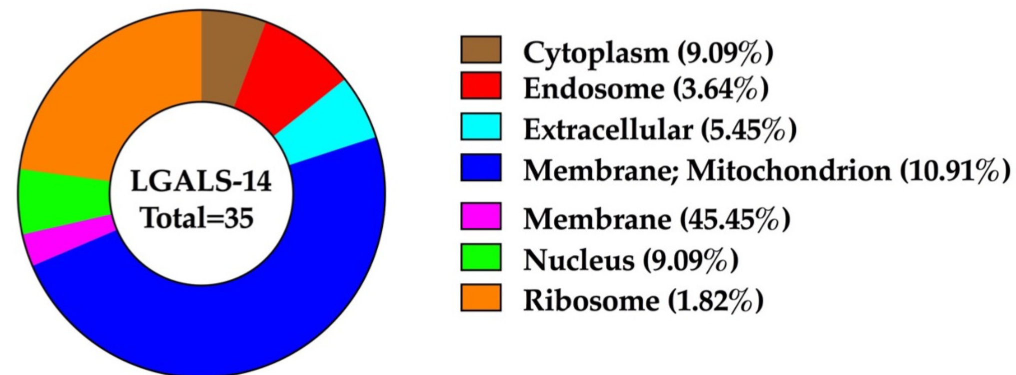


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Figure 5(on next page)

Protein-protein interaction network of adult stage of *Haemonchus contortus* with host galectins.

Protein interactions was determined using the software (SAINT) (Choi et al. 2011) and resulting interaction network was visualised using the Cytoscape v3.4.0.

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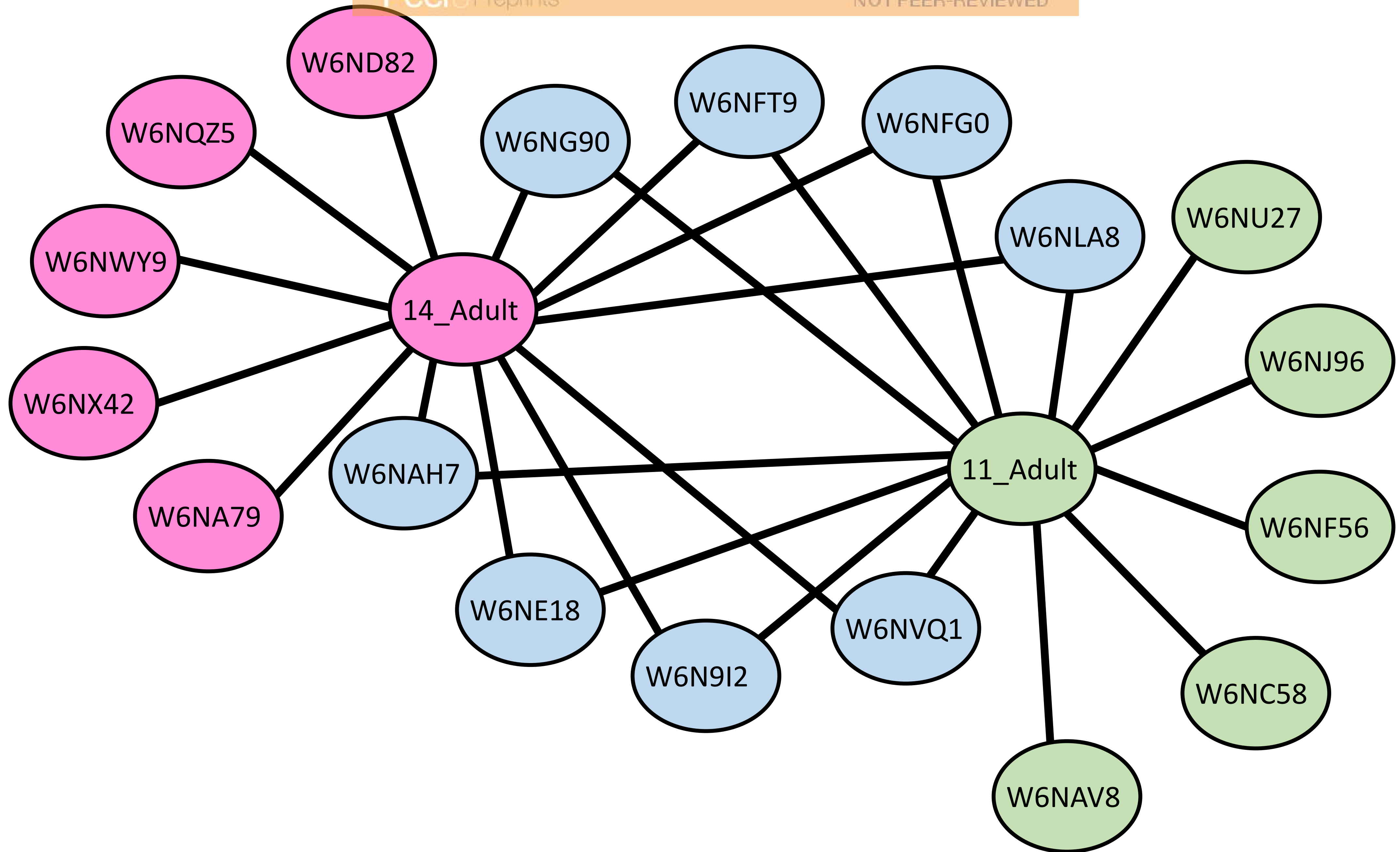

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