Omics tools enabling vaccine discovery against fasciolosis

Krystyna Cwiklinski[1*] and John P. Dalton[2]

1. Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK

2. Molecular Parasitology Laboratory, Centre for One Health (MPL), Ryan Institute, School of Natural Science, National University of Ireland Galway, Galway, Ireland.

K.Cwiklinski ORCID ID: 0000-0001-5577-2735

J.P.Dalton ORCID ID: 0000-0003-3829-9186

*Correspondence: krystyna.cwiklinski@liverpool.ac.uk (K. Cwiklinski).

Keywords: *Fasciola*, helminths, vaccines, genomics, transcriptomics, proteomics.

Abstract

In the past decade significant advances in our understanding of liver fluke biology have been made through in-depth interrogation and analysis of evolving *Fasciola hepatica* and *Fasciola gigantica* omics datasets. This information is crucial for developing novel control strategies, particularly vaccines necessitated by the global spread of anthelmintic resistance. Distilling them down to a manageable number of testable vaccines requires combined rational, empirical, and collaborative approaches. Despite a lack of clear outstanding vaccine candidate(s), we must continue to identify salient parasite-host interacting molecules, likely in the secretory products, tegument, or extracellular vesicles, and perform robust trials especially in livestock, employing present and emerging vaccinology technologies to discover that elusive liver fluke vaccine. Omics tools are bringing this prospect ever closer.

Current control strategies for fasciolosis

Helminth (see **Glossary**) parasite infections impact negatively the health of people worldwide, representing a loss of 10.6 million **disability adjusted life years** each year, and cause major global economic losses within the animal production industry [1, 2]. Fasciolosis, resulting from infection with the liver flukes *Fasciola hepatica* and *Fasciola gigantica*, is now recognised as an important neglected tropical disease contributing substantially to these impacts on humans and their livestock [3]. Current fluke control in both animals and people is reliant on the use of anthelminthic drugs, predominantly triclabendazole (TCBZ). However, due to widespread resistance to TCBZ, climate change factors, intensification of farming and the potential hybridisation of these related parasites [4, 5], the incidence of animal disease is increasing, threatening more human infections. The environmental damage caused by intense farming and parasite control with chemicals is accelerating the need for green preventative measures, particularly vaccines, which alongside improved farm management and diagnostic practices would provide a multi-pronged approach to fasciolosis control.

State of play of vaccines

In their recent review of *Fasciola* spp. vaccines, Spithill and colleagues [6] pointed out that the majority of vaccine trials carried out to-date have concentrated on just a few parasite molecules, namely cathepsin L peptidases, glutathione-S-transferases (GST), fatty acid binding proteins (FABP), and leucine aminopeptidase (LAP), that were of interest because they are abundantly secreted *in vitro* by the mature adult parasites. These vaccine trials first used **native proteins** isolated using conventional biochemical methods from the **excreted/secreted (ES) proteins** of adult parasites but

were later reconfigured as recombinant sub-unit vaccines produced by prokaryotic bacterial and/or eukaryotic yeast expression systems (**Table 1**). Despite promising results reported for some trials (as high as 89% protection against a single challenge infection), in the end, these studies did not identify a vaccine that induces reproducible protection at levels that encouraged further development and translation. Perhaps, therefore, in light of the extensive molecular data for liver fluke that have expanded and evolved as technology improves, we need to re-think our approach to consider a more rational means of choosing vaccine molecules. Even in the short space of time since we reviewed the available *Fasciola* spp. omics data in 2018 [7], the number of available datasets has increased markedly, particularly in relation to those available for *F. gigantica*. A conscious effort is therefore needed to analyse and re-evaluate this collective data to discern novel aspects of liver fluke biology, which will play an important role in vaccine development going forward. Additionally, and/or alternatively, we need to take an empirical approach by screening as many candidates as possible, although the logistics/statistics and high costs of large animal trials (sheep, and more so cattle) negates our ability to perform even medium throughput vaccine screens; it follows, therefore, that these studies will require close collaborations with government/agricultural research institutions with access to appropriate large animal research facilities.

Why is developing a vaccine against liver fluke so difficult?

The growing migratory parasite

The rapid production of the various SARS-CoV-2 vaccines in 2019 was a great achievement and gives optimism for all vaccine development programmes. But this achievement was built upon decades of work on coronaviruses that pinpointed the

spike protein as a prime target for antibody- and cellular-mediated immunity [8, 9]; the spike protein is required for viral cell entry and is also one of only ~30 proteins expressed by the virus. Parasites, particularly the large multicellular helminths, are far more complex than viruses, requiring multiple hosts to complete their life cycle (**Figure 1**), within which they undergo extensive changes in development and growth, expressing thousands of proteins; exemplified by the *Fasciola* spp. parasites that transcribe over 18, 000 genes during their development within the mammalian host [10-12].

Within the mammalian host, the *Fasciola* parasites migrate through several different tissues and cellular environments, involving three major phases (**Figure 1**): (1) The invasion across the intestinal wall by the rapidly moving newly excysted juveniles (NEJ) dependent on endogenous glycogen stores; (2) the migration through the liver **parenchyma** by the immature flukes that undergo extensive growth and development sustained by feeding on host tissues and blood; and (3) the mature stage, where the obligate blood-feeding adult parasites reside in the bile ducts with a primary purpose of producing thousands of eggs per day. Analysis by histopathology [13] has shown that active locomotion and migration ensures that the parasites elude the onslaught of rapidly-recruited host immune cells, especially eosinophils, macrophages and lymphocytes; a response that is also needed to repair the extensive damage caused by the parasites' tunnelling. These cells are undoubtedly attracted by the many antigens released from the parasite **tegument**, gut and reproductive organs via **classical, non-classical secretion** pathways and as cargo of **extracellular vesicles** [13], and also by host **alarmins** released from damaged tissue [14].

No protective immunity displayed during natural infection

Demonstration of natural immunity to infectious organisms augurs well for the downstream development of a protective vaccine. But, in this regard, there is a paucity of information relating to the ability of *F. hepatica* to induce protective innate and cellular immunity during infections particularly in sheep and cattle, unlike comparable studies of gastrointestinal nematode infection of ruminants [15, 16]. Studies in sheep generally conclude that protection is not elicited during primary or challenge infection, with the exception of Indonesian thin-tail (ITT) sheep that elicit high levels of immune-mediated resistance to infection by *F. gigantica* but not *F. hepatica* [16-19]. Resistance to re-infection shown in cattle is thought to be related to extensive liver fibrosis that occurs during primary infection, rather than this being immune-mediated [16, 17]. Although studies by Hoyle et al. [20] indicated that drug abbreviated infections can induce some protection against liver damage in cattle, field studies have shown that despite exposure to multiple natural infections while out on pasture, ruminants are still susceptible to further infections [21, 22]. This underlines the need for more studies on liver fluke infections in ruminants to identify robust markers and mechanisms of protection. Analysis of antibody responses to infections in ruminants is highly polarised towards IgG1 classes, indicative of a potent Th2-driven immune response to infection [23], and vaccine studies suggest that partial protection is associated with the induction of IgG2 antibodies [6, 22]. But, whether IgG2 antibodies are protective in themselves or are markers of a broader (Th1-driven) immune response needs further clarification. Nevertheless, to-date vaccine-induced IgG2 is the only suggested biomarker of potential natural and vaccine-induced protection proposed so far, underscoring the need for more research in this area.

Modulation of the host immune responses

A well-known feature of liver fluke infection in laboratory animals and ruminants is the rapid skewing of the host immune response from an early Th1/Th2 response towards a potent Th2 and hyporesponsive phenotype [16]; a response that is essential for wound healing but may not offer any protection against liver fluke parasites [24]. Th2-driven responses induced by helminths can impair immune responses of bacterial co-infections [25-27] and vaccine-induced Th1 performance (e.g. schistosomes and diphtheria, hepatitis B and tetanus vaccines; [28-30]) and therefore needs to be considered for liver fluke vaccine development as challenge infections can negate the effects of the vaccination-elicited responses. A range of parasite proteins found in the parasite **secretomes** have been shown to be immunomodulatory, including the cathepsin peptidases, helminth defense molecule (FhHDM), Kunitz-like inhibitor (FhKT1), peroxiredoxin (FhPrx), GSTs, and FABPs [31]. As such, this presents a good example of a rational approach to vaccine development, i.e. targeting important parasite immunomodulators, as these molecules could induce responses that counteract the parasite's ability to control host immune responses [31]. Further studies on molecules involved in immunomodulation by liver flukes is therefore highly warranted.

Interrogation and integration of omics data is advancing our knowledge of liver fluke biology

Deciphering the functional role that every parasite molecule plays at each phase of the life cycle is critical for the future development of new control strategies. The advances in sequencing technologies and their application for analysis of *Fasciola* species over the past decade has laid the foundation for a global molecular view of

parasite developmental biology. Detailed genomic, transcriptomic and proteomic/glycomic data has given us a complete picture from the starting DNA through to the expression, production and post-translational modification of many critical parasite proteins that can be exploited for the development of novel control strategies (**Box 1**; [32]).

For both *Fasciola* spp., multiple genome assemblies are available from samples collected in geographical disparate locations; *F. hepatica* isolates from the UK and USA, and *F. gigantica* isolates from Uganda, India and China. These genome assemblies highlight several multi-membered gene families that are known to play important roles in the *Fasciola* spp., such as the papain-like cysteine peptidases, cysteine peptidases inhibitors, G protein-coupled receptors (GPCRs) and a plethora of kinases (**Box 2**; [32, 33]). The availability of these datasets now allows genome-wide comparative studies to investigate how liver flukes and their biological strategies have evolved, recently revealing the evolutionary divergence of the *Fasciola* spp. 5 million years ago [34]. By extension, we can now investigate how both parasites have since evolved and adapted to infect a wide range of hosts and determine what role genetic diversity plays in parasite virulence and survival.

Comprehensive transcriptome analyses from multiple life cycle stages (seven *F. hepatica* life cycle stages [10, 12, 35, 36] and eight *F. gigantica* life cycle stages [37]), provide information as to when and where these important genes are being turned on and off. These studies highlight the complex and dynamic changes the parasites undergo as they transition through their developmental stages within the different environments in the host. Differential gene transcription analysis of the *Fasciola* spp.

stage-specific transcriptomes shows that distinct subsets of genes are expressed by the parasite as it migrates through the host [10, 37]. This strict gene regulation is controlled in part by non-coding RNAs, of which the majority of our knowledge pertains to the regulatory microRNAs (miRNAs) that also display temporal expression throughout the life cycle [32, 38-40]. Amongst the most highly regulated genes are those found within large gene families, specifically the secreted cathepsin cysteine peptidases and the structural tubulins comprising alpha and beta tubulin, that play stage-specific roles (**Box 2**; [10]).

A significant advancement in our knowledge of how the parasite interacts with its host has been driven by our ability to analyse the parasite stages involved in early infection, namely the NEJ and immature flukes, that were previously challenging to analyse due to difficulty in obtaining sufficient parasite material [11, 12]. Underpinned by the regulation of a large number of genes following the development from NEJ to mature adults (up-regulation of ~18,000 genes), proteomic studies highlight the temporal expression of parasite molecules that are required as the parasite migrates through different tissues and microenvironments within the host [11, 12, 41]. High expression of pathways associated with carbohydrate metabolism and signal transduction reveal the metabolic changes driving parasite growth and development as the NEJ transitions from aerobic metabolism to the reliance on aerobic acetate production displayed by the immature flukes in the liver [10-12]. Analysis of the ES proteins reveals the array of secreted molecules that are important for parasite survival amongst which are a diverse range of proteases, protease inhibitors, antioxidants, immunomodulators, metabolic enzymes (and a number of uncharacterised proteins) [11, 12, 32, 41-44]. These proteins are involved in critical processes including the transition to blood

feeding (cathepsin peptidases, saposins, LAP), protease regulation (protease inhibitors) and immunomodulation/evasion (as described above). Investigation of the role these proteins play is being supported by **immunolocalisation** of the proteins within NEJ and adult fluke sections, and biochemical analyses of recombinantly expressed proteins provides insight into their biological function.

Omics-inspired anti-fluke vaccine development

Vaccine development against fasciolosis has gradually focussed on those molecules present at the host-parasite interface, specifically proteins within the parasite tegument and gut, and now extracellular vesicles, which appear in the parasite secretome and play a critical role in host invasion, host immune modulation/manipulation and parasite survival. Proteomic studies have increased our understanding of these important proteins by unmasking their expression and secretory profiles in the various *F. hepatica* developmental life cycle stages, namely NEJ (159 proteins; [11]), immature flukes (210 proteins; [12]) and adult flukes (227; [12]).

Proteases dominate the secretomes, specifically the cathepsin cysteine peptidases that are involved in migration, blood feeding and immunomodulation [45]. Since all these functions must be highly regulated to prevent excessive host damage, inhibitors of these parasite proteases (stefins and Kunitz-type inhibitors) are also abundantly secreted, particularly by the liver-migrating immature flukes [11, 12]. But some secreted inhibitors do not have known targets within the parasite, e.g. serine protease inhibitors (serpins), and thus are suspected to have been adapted to control host proteases such a lysosome-associated cathepsins [46]. Thereby, they impair antigen processing in innate immune cells such as macrophages, and impede the action of

serum-related proteases (e.g. thrombin, plasmin, kininogen and MBL-associated serine proteases; MASPs) involved in the initiation of the Lectin complement pathway and blood coagulation [46, 47]. Omics analyses have also revealed that a plethora of antioxidants, such as thioredoxin (FhTrx), FhPrx, superoxide dismutases (FhSODs) and GSTs, are predominately secreted by the NEJ and adult stage parasites [11, 12, 42, 48]. Collectively, these molecules defend the parasite against the damaging effects of various **reactive oxygen species (ROS)**, but also have moonlighting roles in the modulation and regulation of the host immune response, such as the alternative activation of M2 macrophages by FhPrx [48, 49].

The *F. hepatica* secretome contains a variety of extracellular vesicles (EVs), whose cargo (proteins, lipids and miRNAs) interact and manipulate the host immune response following EV internalisation by host cells [50]. EVs that are predominantly released from cells surrounding the gastrodermis in adult parasites [51, 52], are also released by several life cycle stages, including eggs and NEJ [53], indicating that EVs play a stage-specific role. We and others discovered that EVs are an important route for the exportation of proteins that lack a signal peptide for classical secretion, such as the abundantly expressed antioxidant FhTrx, various metabolic enzymes (enolase, fructose-bisphosphate aldolase) and the Kunitz-type protease inhibitor [44, 54, 55]. Given the immunomodulatory role of parasite EVs, they have been proposed as vaccine candidates [56], though the utility of this strategy has yet to be demonstrated for fasciolosis. Targeting exposed epitopes on EV surface proteins and glycoproteins clearly represent prime targets for vaccines.

Recent characterisation of protein post-translational modifications namely glycosylation has revealed that the *F. hepatica* glycome is also dynamic and stage-specific [32]. *F. hepatica* glycans are displayed on the parasite outer surface, in addition to being released into the host environment on the EVs and the secreted proteins. They are a key player in host-parasite interactions, mimicking host glycans and promoting the maturation of dendritic cells driving Th2 type immune responses and preventing the activation of complement [32, 47]. While we have only begun to scratch the glycan surface of *F. hepatica*, elucidation of the role they play will be important for future vaccine design, either as direct targets or as accompanying **adjuvants** in cocktail vaccines.

Multi-antigen vaccine for a multicellular organism

Despite our increased knowledge of the complexity of the *Fasciola* spp. parasites, few early *F. hepatica* vaccine studies embraced a multi-target approach based on more than one parasite protein or functional type of protein; an approach that has been pursued by researchers involved in the EU consortia PARAVAC (grant id 265862) and PARAGONE (grant id 635408) battling various worm parasites of ruminants [57, 58].

Since the release of the first draft *F. hepatica* genome in 2015 [10], only five studies have employed a multi-antigen vaccine approach for control of fasciolosis, using combinations of potentially functionally different proteins (FhGST and tegumental antigen [59]; FhLAP and FhCL1 [60]; FhTeg1 and FhTeg5 [61]; FhCL1, FhPrx, FhLAP, FhHDM [58]; FhStf1, FhStf2, FhStf3, FhKT1 [62]). These studies reported varying levels of vaccine efficacy, based on reductions of adult parasites and eggs. Surprisingly, when compared to vaccine trials using single antigens, these

combination vaccines appear were less effective (**Table 1**), although direct comparisons between trials is tentative due to the inconstancies in experimental design, including the host animal used for the trial, the number of animals per group and the adjuvant used for vaccine formulation. This also highlights the need for consistent methods of vaccine efficacy assessment, which should not solely rely on mean fluke burdens but should also address the effects of liver fluke infection on animal health and welfare that impacts animal productivity [6, 62, 63]. Nevertheless, it may signal that more antigens in vaccine cocktails is not necessarily better than individual antigens and raises doubts concerning which combination of antigens should be used and how we assess the contribution of each individual antigen in vaccine efficacy versus their amalgamated effect.

Targeting the NEJ

Recent vaccine strategies are aimed at preventing the damaging effects of the parasite's invasion and migration through the liver tissues rather than focussing on molecules released by the adult parasites that represent long-established infections [62, 64]. Moreover, once in the liver the rapid growth, development and continuous movement of the parasite makes the immune response of the host rather ineffectual as the parasite has moved on (with the exception of the immune responses observed in *F. gigantica*-infected ITT sheep). Targeting the NEJ as it enters the host via the intestine and before it enters the liver makes sense and dissection of the molecular make-up of the NEJ tegument and secretome has revealed that the NEJ express and secrete a unique array of proteins (e.g. a family of cathepsin B peptidases and a cathepsin L3 peptidase with unique collagenase-like substrate specificity [45]) compared with the liver and adult stages [11, 12].

297

298 Functional biochemical and molecular characterisation analyses are beginning to
299 reveal the potential redundancy in biological function of the proteins secreted by *F.*
300 *hepatica*, whereby multiple proteins and/or mechanisms are employed to modulate
301 host proteins; for example, the use of stefins and Kunitz-type inhibitors to modulate
302 cathepsin peptidases [62, 65, 66]. We have categorised these proteins into key
303 biological processes (e.g. proteases, protease inhibitors, antioxidants,
304 immunomodulators) that can be combined within a cocktail vaccine that may offer a
305 strategy to overcome this molecular redundancy that could block or interfere with key
306 parasite processes to more effectively combat these multicellular organisms.
307 Clarification of these protein families using the *Fasciola* spp. genomic platforms has
308 facilitated the identification of stage-specific members, especially those that NEJ-
309 specific, which aid our selection of vaccine candidates.

310

311 **Concluding remarks**

312 Our interrogation of these large sequencing datasets has provided novel insights into
313 how *F. hepatica* and *F. gigantica* navigate the various host tissues and environments
314 they encounter. Moving forward, we now need to mirror our omics understanding of
315 liver fluke infection from the host's point of view to provide a comparative global view
316 of the dynamics of host-parasite interactions. But the process of deciphering what
317 these *Fasciola* spp. datasets can reveal about the parasite's biology and relationship
318 with its hosts has only just begun (see **Outstanding questions**) since recent analysis
319 of our datasets has revealed that a vast number of the predicted proteins derived from
320 these genome datasets remain uncharacterised, some of which must play an
321 important role during the invasion and migratory phases of the life cycles.

Omics studies can now provide a complete menu of candidates that can be used to test new ideas of vaccination. Despite the slow rate of progress to date, continued evaluation of new vaccination strategies (e.g. different adjuvants, formulations, delivery; [67]), informed by immunoinformatic/immunoproteomic approaches such as described by [68, 69], is necessary to achieve a breakthrough in the development of fasciolosis vaccines. Importantly, robust immune response and vaccine efficacy analyses is required, which necessitates using relevant target host species (sheep, cattle, goats, buffalo), suitable numbers of animals and appropriately repeated experiments to ensure statistical robustness, a major logistical, experimental and financial challenge in itself.

Acknowledgements

Science Foundation Ireland (SFI) Professorship grant (17/RP/5368) awarded to JPD.

Declaration of Competing Interest

The authors declare that they have no competing interests.

References

1. GBD 2019 Diseases and Injuries Collaborators (2020) Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396 (10258), 1204-1222.

2. Charlier, J. et al. (2020) Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. Prev Vet Med 182, 105103.

3. Lalor, R. et al. (2021) Pathogenicity and virulence of the liver flukes *Fasciola hepatica* and *Fasciola gigantica* that cause the zoonosis fasciolosis. Virulence 12 (1), 2839-2867.
4. Fairweather, I. et al. (2020) Drug resistance in liver flukes. Int J Parasitol Drugs Drug Resist 12, 39-59.
5. Knubben-Schweizer, G. et al. (2022) Epidemiology and Control. In Fasciolosis (Dalton, J.P. ed), pp. 180-210, CAB International.
6. Spithill, T. et al. (2022) Vaccines for *Fasciola*: New thinking for an old problem. In Fasciolosis (Dalton, J.P. ed), pp. 379-422, CAB International.
7. Cwiklinski, K. and Dalton, J.P. (2018) Advances in *Fasciola hepatica* research using 'omics' technologies. IntJ Parasitol 48 (5),321-331.
8. Dai, L. and Gao, G.F. (2021) Viral targets for vaccines against COVID-19. Nat Rev Immunol 21 (2), 73-82.
9. Cox, R.J. and Brokstad, K.A. (2020) Not just antibodies: B cells and T cells mediate immunity to COVID-19. Nat Rev Immunol 20 (10), 581-582.
10. Cwiklinski, K. et al. (2015) The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. Genome Biol 16, 71-015-0632-2.
11. Cwiklinski, K. et al. (2018) Infection by the helminth parasite *Fasciola hepatica* requires rapid regulation of metabolic, virulence, and invasive factors to adjust to its mammalian host. Mol Cell Proteomics 17 (4), 792-809.
12. Cwiklinski, K. et al. (2021) Complementary transcriptomic and proteomic analyses reveal the cellular and molecular processes that drive growth and development of *Fasciola hepatica* in the host liver. BMC Genomics 22 (1), 46.
13. Sangster, N.C. et al. (2022) Pathology, pathophysiology and clinical aspects. In Fasciolosis (Dalton, J.P. ed), pp. 145-179, CAB International.
14. Robinson, M.W. et al. (2010) Worm secretory molecules are causing alarm. Trends Parasitol 26 (8), 371-372.
15. Britton, C. et al. (2020) The potential for vaccines against scour worms of small ruminants. Int J Parasitol 50 (8), 533-553.
16. Donnelly, S. et al. (2022) Immunological interaction between *Fasciola* and its host. In Fasciolosis (Dalton, J.P. ed), pp. 278-307, CAB International.
17. Molina-Hernandez, V. et al. (2015) *Fasciola hepatica* vaccine: we may not be there yet but we're on the right road. Vet Parasitol 208 (1-2), 101-111.

18. Piedrafita, D. et al. (2004) Immunology of the host-parasite relationship in fasciolosis (*Fasciola hepatica* and *Fasciola gigantica*). Can J Zool 82, 233-250.
19. Pleasance, J. et al. (2011) Resistance to liver fluke infection in the natural sheep host is correlated with a type-1 cytokine response. Parasite Immunol 33 (9), 495-505.
20. Hoyle, D.V. et al. (2003) Pre-exposure of cattle to drug-abbreviated *Fasciola hepatica* infections: the effect upon subsequent challenge infection and the early immune response. Vet Parasitol 111 (1), 65-82.
21. Dalton, J.P. and Mulcahy, G. (2001) Parasite vaccines-a reality? Vet Parasitol 98 (1-3), 149-167.
22. Mulcahy, G. et al. (1998) Correlation of specific antibody titre and avidity with protection in cattle immunized against *Fasciola hepatica*. Vaccine 16 (9-10), 932-939.
23. Clery, D. et al. (1996) Immune responses of chronically infected adult cattle to *Fasciola hepatica*. Vet Parasitol 62 (1-2), 71-82.
24. Gause, W.C. et al. (2013) Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Nat Rev Immunol 13 (8), 607-14.
25. Brady, M.T. et al. (1999) *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. Infect Immun 67 (10), 5372-5378.
26. O'Neill, S.M. et al. (2001) *Fasciola hepatica* cathepsin L cysteine proteinase suppresses *Bordetella pertussis*-specific interferon-gamma production *in vivo*. Parasite Immunol 23 (10), 541-547.
27. Naranjo Lucena, A. et al. (2017) The immunoregulatory effects of co-infection with *Fasciola hepatica*: From bovine tuberculosis to Johne's disease. Vet J 222, 9-16.
28. Riner, D.K. et al. (2016) *Schistosoma mansoni* infection can jeopardize the duration of protective levels of antibody responses to immunizations against hepatitis B and tetanus toxoid. PLoS Negl Trop Dis 10 (12), e0005180.
29. Haseeb, M.A. and Craig, J.P. (1997) Suppression of the immune response to diphtheria toxoid in murine schistosomiasis. Vaccine 15 (1), 45-50.
30. Wait, L.F. et al. (2020) Do parasite infections interfere with immunisation? A review and meta-analysis. Vaccine 38 (35), 5582-5590.
31. Ryan, S. et al. (2020) *Fasciola hepatica*-derived molecules as regulators of the host immune response. Front Immunol 11, 2182.

32. Cwiklinski, K. et al. (2022) Applying 'Omics' technologies to understand *Fasciola* spp. biology. In Fasciolosis (Dalton, J.P. ed), pp. 338-378, CAB International.
33. International Helminth Genomes Consortium. (2019) Comparative genomics of the major parasitic worms. Nat Genet 51 (1), 163-174.
34. Choi, Y.J. et al. (2020) Adaptive radiation of the flukes of the family Fasciolidae inferred from genome-wide comparisons of key species. Mol Biol Evol 37 (1), 84-99.
35. Ilgová, J. et al. (2022) Transcriptomic and proteomic profiling of peptidase expression in *Fasciola hepatica* eggs developing at host's body temperature. Sci Rep 12 (1), 10308.
36. McNulty, S.N. et al. (2017) Genomes of *Fasciola hepatica* from the Americas reveal colonization with neorickettsia endobacteria related to the agents of potomac horse and human sennetsu fevers. PLoS genetics 13 (1), e1006537.
37. Zhang, X.Q. et al. (2019) Complex and dynamic transcriptional changes allow the helminth *Fasciola gigantica* to adjust to its intermediate snail and definitive mammalian hosts. BMC Genomics 20 (1), 729.
38. Ricafrente, A. et al. (2022) Stage-specific miRNAs regulate gene expression associated with growth, development and parasite-host interaction during the intra-mammalian migration of the zoonotic helminth parasite *Fasciola hepatica*. BMC Genomics 23 (1), 419.
39. Herron, C.M. et al. (2022) Developmental regulation and functional prediction of microRNAs in an expanded *Fasciola hepatica* miRNome. Front Cell Infect Microbiol 12, 811123.
40. Fontenla, S. et al. (2021) Role of *Fasciola hepatica* small RNAs in the interaction with the mammalian host. Front Cell Infect Microbiol 11, 812141.
41. Di Maggio, L.S. et al. (2016) Across intra-mammalian stages of the liver fluke *Fasciola hepatica*: a proteomic study. Sci Rep 6, 32796.
42. Murphy, A. et al. (2020) *Fasciola hepatica* extracellular vesicles isolated from excretory-secretory products using a gravity flow method modulate dendritic cell phenotype and activity. PLoS Negl Trop Dis 14 (9), e0008626.
43. Di Maggio, L.S. et al. (2019) A proteomic comparison of excretion/secretion products in *Fasciola hepatica* newly excysted juveniles (NEJ) derived *from Lymnaea viatrix* or *Pseudosuccinea columella*. Exp Parasitol 201, 11-20.

448 44. Cwiklinski, K. et al. (2015) The extracellular vesicles of the helminth pathogen,
449 *Fasciola hepatica*: Biogenesis pathways and cargo molecules involved in parasite
450 pathogenesis. Mol Cell Proteomics 14 (12), 3258-3273.
451 45. Cwiklinski, K. et al. (2019) The cathepsin-like cysteine peptidases of trematodes
452 of the genus *Fasciola*. Adv Parasitol 104, 113-164.
453 46. De Marco Verissimo, C. et al. (2020) *Fasciola hepatica* serine protease inhibitor
454 family (serpins): Purposely crafted for regulating host proteases. PLoS Negl Trop Dis
455 14 (8), e0008510.
456 47. De Marco Verissimo, C. et al. (2022) *Fasciola hepatica* is refractory to
457 complement killing by preventing attachment of mannose binding lectin (MBL) and
458 inhibiting MBL-associated serine proteases (MASPs) with serpins. PLoS Pathog 18
459 (1), e1010226.
460 48. Dorey, A. et al. (2021) Autonomous non-antioxidant roles for *Fasciola hepatica*
461 secreted thioredoxin-1 and peroxiredoxin-1. Front Cell Infect Microbiol 11, 667272.
462 49. Donnelly, S. et al. (2005) Thioredoxin peroxidase secreted by *Fasciola hepatica*
463 induces the alternative activation of macrophages. Infect Immun 73 (1), 166-173.
464 50. Sotillo, J. et al. (2020) The protein and microRNA cargo of extracellular vesicles
465 from parasitic helminths - current status and research priorities. Int J Parasitol 50 (9),
466 635-645.
467 51. Bennett, A.P.S. et al. (2020) The cellular and molecular origins of extracellular
468 vesicles released by the helminth pathogen, *Fasciola hepatica*. Int J Parasitol 50 (9),
469 671-683.
470 52. Bennett, A.P.S. et al. (2022) *Fasciola hepatica* gastrodermal cells selectively
471 release extracellular vesicles via a novel atypical secretory mechanism. Int J Mol Sci
472 23 (10).
473 53. Trelis, M. et al. (2022) Proteomic analysis of extracellular vesicles from *Fasciola*
474 *hepatica* hatching eggs and juveniles in culture. Front Cell Infect Microbiol 12,
475 903602.
476 54. Marcilla, A. et al. (2012) Extracellular vesicles from parasitic helminths contain
477 specific excretory/secretory proteins and are internalized in intestinal host cells. PloS
478 one 7 (9), e45974.
479 55. de la Torre-Escudero, E. et al. (2019) Surface molecules of extracellular vesicles
480 secreted by the helminth pathogen *Fasciola hepatica* direct their internalisation by
481 host cells. PLoS Negl Trop Dis 13 (1), e0007087.

482 56. Drurey, C. et al. (2020) Extracellular vesicles: new targets for vaccines against
483 helminth parasites. Int J Parasitol 50 (9), 623-633.
484 57. Nisbet, A.J. et al. (2019) The rational simplification of a recombinant cocktail
485 vaccine to control the parasitic nematode *Teladorsagia circumcincta*. Int J Parasitol
486 49 (3-4), 257-265.
487 58. Zafra, R. et al. (2021) Efficacy of a multivalent vaccine against *Fasciola hepatica*
488 infection in sheep. Vet Res 52 (1), 13.
489 59. Zerna, G. et al. (2022) Bovine natural antibody relationships to specific
490 antibodies and *Fasciola hepatica* burdens after experimental infection and
491 vaccination with glutathione S-transferase. Vet Sci 9 (2).
492 60. Ortega-Vargas, S. et al. (2019) Moderate protection is induced by a chimeric
493 protein composed of leucine aminopeptidase and cathepsin L1 against *Fasciola*
494 *hepatica* challenge in sheep. Vaccine 37 (24), 3234-3240.
495 61. McCusker, P. et al. (2020) Molecular characterisation and vaccine efficacy of two
496 novel developmentally regulated surface tegument proteins of *Fasciola hepatica*. Vet
497 Parasitol 286, 109244.
498 62. Cwiklinski, K. et al. (2022) Targeting secreted protease/anti-protease balance as
499 a vaccine strategy against the helminth *Fasciola hepatica*. Vaccines (Basel) 10 (2).
500 63. Charlier, J. et al. (2014) Recent advances in the diagnosis, impact on production
501 and prediction of *Fasciola hepatica* in cattle. Parasitol 141 (3), 326-335.
502 64. González-Miguel, J. et al. (2021) Insights into *Fasciola hepatica* juveniles:
503 crossing the Fasciolosis rubicon. Trends Parasitol 37 (1), 35-47.
504 65. Smith, D. et al. (2016) Unexpected activity of a novel Kunitz-type inhibitor:
505 inhibition of cysteine proteases but not serine proteases. J Biol Chem 291 (37),
506 19220-19234.
507 66. Smith, D. et al. (2020) An atypical and functionally diverse family of Kunitz-type
508 cysteine/serine proteinase inhibitors secreted by the helminth parasite *Fasciola*
509 *hepatica*. Sci Rep 10 (1), 20657.
510 67. Entrican, G. and Francis, M.J. (2022) Applications of platform technologies in
511 veterinary vaccinology and the benefits for one health. Vaccine 40 (20), 2833-2840.
512 68. Garza-Cuartero, L. et al. (2018) Antibody recognition of cathepsin L1-derived
513 peptides in *Fasciola hepatica*-infected and/or vaccinated cattle and identification of
514 protective linear B-cell epitopes. Vaccine 36 (7), 958-968.

69. Cameron, T.C. et al. (2017) A novel ex vivo immunoproteomic approach characterising *Fasciola hepatica* tegumental antigens identified using immune antibody from resistant sheep. Int J Parasitol 47 (9), 555-567.
70. Pandey, T. et al. (2020) Draft genome of the liver fluke *Fasciola gigantica*. ACS Omega 5 (19), 11084-11091.
71. Luo, X. et al. (2021) High-quality reference genome of *Fasciola gigantica*: Insights into the genomic signatures of transposon-mediated evolution and specific parasitic adaption in tropical regions. PLoS Negl Trop Dis 15 (10), e0009750.
72. Fontenla, S. et al. (2015) The miRnome of *Fasciola hepatica* juveniles endorses the existence of a reduced set of highly divergent micro RNAs in parasitic flatworms. Int J Parasitol 45 (14), 901-913.
73. Hu, R.S. et al. (2021) Differential expression of microRNAs and tRNA fragments mediate the adaptation of the liver fluke *Fasciola gigantica* to its intermediate snail and definitive mammalian hosts. Int J Parasitol 51 (5), 405-414.
74. González-Miguel, J. et al. (2020) Set up of an *in vitro* model to study early host-parasite interactions between newly excysted juveniles of *Fasciola hepatica* and host intestinal cells using a quantitative proteomics approach. Vet Parasitol 278, 109028.
75. Davis, C.N. et al. (2019) The importance of extracellular vesicle purification for downstream analysis: A comparison of differential centrifugation and size exclusion chromatography for helminth pathogens. PLoS Negl Trop Dis 13 (2), e0007191.
76. Morphew, R.M. et al. (2007) Comparative proteomics of excretory-secretory proteins released by the liver fluke *Fasciola hepatica* in sheep host bile and during in vitro culture ex host. Mol Cell Proteomics 6 (6), 963-972.
77. Wilson, R.A. et al. (2011) Exploring the *Fasciola hepatica* tegument proteome. Int J Parasitol 41 (13-14), 1347-1359.
78. Hacariz, O. et al. (2012) A proteomic approach to investigate the distribution and abundance of surface and internal *Fasciola hepatica* proteins during the chronic stage of natural liver fluke infection in cattle. J Proteome Res 11 (7), 3592-3604.
79. Hacariz, O. et al. (2014) Generating a detailed protein profile of *Fasciola hepatica* during the chronic stage of infection in cattle. Proteomics 14 (12), 1519-1530.
80. Morphew, R.M. et al. (2013) Identification of the major proteins of an immune modulating fraction from adult *Fasciola hepatica* released by Nonidet P40. Vet Parasitol 191 (3-4), 379-385.

549 81. Ravida, A. et al. (2016) *Fasciola hepatica* surface tegument: Glycoproteins at the
550 interface of parasite and host. Mol Cell Proteomics 15 (10), 3139-3153.
551 82. Hu, R.S. et al. (2020) Proteomic profiling of the liver, hepatic lymph nodes, and
552 spleen of buffaloes infected with *Fasciola gigantica*. Pathogens 9 (12).
553 83. Zhang, F.K. et al. (2019) Global serum proteomic changes in water buffaloes
554 infected with *Fasciola gigantica*. Parasit Vectors 12 (1), 281.
555 84. Garcia-Campos, A. et al. (2016) Tegument glycoproteins and cathepsins of
556 newly excysted juvenile *Fasciola hepatica* carry mannosidic and paucimannosidic N-
557 glycans. PLoS Negl Trop Dis 10 (5), e0004688.
558 85. Ravida, A. et al. (2016) *Fasciola hepatica* surface coat glycoproteins contain
559 mannosylated and phosphorylated N-glycans and exhibit immune modulatory
560 properties independent of the mannose receptor. PLoS Negl Trop Dis 10 (4),
561 e0004601.
562 86. Pan, M. et al. (2022) A global phosphoproteomics analysis of adult *Fasciola*
563 *gigantica* by LC-MS/MS. Parasitol Res 121 (2), 623-631.
564 87. Hu, G.M. et al. (2017) Visualizing the GPCR Network: Classification and
565 Evolution. Sci Rep 7 (1), 15495.
566 88. McVeigh, P. et al. (2018) Profiling G protein-coupled receptors of *Fasciola*
567 *hepatica* identifies orphan rhodopsins unique to phylum Platyhelminthes. Int J
568 Parasitol Drugs Drug Resist 8 (1), 87-103.
569 89. Cooper, G.M. (2000) Microtubules. In The Cell: A Molecular Approach (2nd
570 edition edn), https://www.ncbi.nlm.nih.gov/books/NBK9932/, Sinauer Associates.
571 90. Fennell, B. et al. (2008) Microtubules as antiparasitic drug targets. Expert Opin
572 Drug Discov 3 (5), 501-18.
573 91. Gasic, I. (2022) Regulation of tubulin gene expression: from isotype identity to
574 functional specialization. Front Cell Dev Biol 10, 898076.
575 92. Toet, H. et al. (2014) Liver fluke vaccines in ruminants: strategies, progress and
576 future opportunities. Int J Parasitol 44 (12), 915-27.

577

578 **Glossary**

579 **Adjuvant**: substance added to the vaccine antigens to increase/modulate the
580 efficacy/potency of the immune response to the vaccine components.

Alarmin: molecules (proteins/peptides) released following cellular damage that signals the immune system to respond.

Classical/Non-classical secretion: Proteins involved in classical secretion contain a secretory signal peptide sequence that directs the protein to endoplasmic reticulum (ER) and Golgi apparatus for transport across the cell plasma membrane. Proteins that do not contain a signal peptide i.e. are leaderless, follow the non-classical route of secretion that does not involved the ER-Golgi system. There is no one mechanism for non-classical secretion.

Disability adjusted life years: measure of disease burden, based on the number of years lost due to ill-health, disability or early death.

Extracellular vesicles: non-replicating vesicles surrounded by a lipid membrane that contain proteins, nucleic acids (DNA, microRNAs etc), lipids and sugars (glycans) that are release by most cell types and are involved in cell to cell communication.

Fasciolosis: disease caused by infection with the helminth parasites *Fasciola hepatica* and *Fasciola gigantica*.

Helminth: invertebrate worms characterised by elongated, flat or round bodies.

Immunolocalisation: Process of detecting proteins in tissue samples using antibodies.

Native protein: Proteins purified from their natural source, which are properly folded and fully functional protein.

Omics: field of study relating to the collective characterisation of biological molecules (genome - DNA, transcript - RNA, proteins, lipids, sugars etc). The individual fields end with the suffix -omics (genomics, transcriptomics, proteomics etc).

Parenchyma: functional tissue within an organ or organism.

Reactive oxygen species (ROS): molecules and free radicals derived from molecular oxygen that are released from molecular oxygen during oxidative metabolism and as a cellular response to drugs, cytokines, tissue/cellular damage and pathogen invasion.

Secretome / excreted/secreted proteins (ES): collection of molecules (proteins, microRNAs, extracellular vesicles) released by live helminths from the parasite tegument, gut and reproductive organs by active secretion or passive release.

Tegument: dynamic cellular structure that covers the surface of organisms within the Phylum Platyhelminthes, specifically tapeworms (cestodes) and flukes (trematodes).

Box 1. Advances in *Fasciola* spp. omics datasets

Over the last decade significant advances have been made in the availability of omics datasets for the *Fasciola* spp. These omics data are freely available to the liver fluke community; highlighted here is what is currently available and where it is housed (**Figure I**).

Genomics: The *Fasciola* spp. data available at WormBase ParaSite (https://parasite.wormbase.org) includes (1) *F. hepatica* genome sequence data from an UK isolate (PRJEB6687, PRJEB25283; [10]) and isolates from the Americas (PRJNA179522; [34, 36]), and (2) *F. gigantica* genome sequence data from an isolate from Uganda (PRJNA230515; [34]). Two further *F. gigantica* genome datasets are also available for isolates from India (NCBI: MKHB03000000; [70]) and China (NCBI: PRJNA691688, Genome Warehouse: GWHAZTT00000000; [71]).

Transcriptomics: Comprehensive transcriptome analyses have been carried out on several *Fasciola* spp. developmental life cycle stages; available datasets include: (3) *F. hepatica* transcriptomes from eggs (NCBI Gene Expression Omnibus: GSE160622; [35]), metacercariae, NEJ 1hr, NEJ 3hr, NEJ 24hr, Immature fluke and adult parasites (NCBI/ENA: PRJEB6904, PRJNA665699; [10, 12] and (4) *F. gigantica* developmental life cycle stage-specific transcriptomes, namely egg, miracidia, rediae, cercariae, metacercariae, immature flukes at 42 days post infection (dpi) and 70 dpi, and adult parasites (NCBI/ENA: PRJNA350370; [37]).

Small non-coding RNAs (microRNA; miRNAs): MicroRNA datasets are available for the following (5) *F. hepatica* life cycle stages metacercariae, NEJ, immature fluke, adult parasites and extracellular vesicles from NEJ and adult flukes generated from three studies (SRR3584125, SRR3584124, SRR3584126, SRR3584122,

638 SRS862512, PRJNA782636 [40, 72]; PRJEB48810 [39]; GSE186948 [38]) and (6) *F.*
639 *gigantica* life cycle stages described above [73].

640 Proteomics: Datasets have been generated for *F. hepatica* proteins derived from the
641 somatic proteome, tegument and ES proteins (secretome) [11, 12, 32, 35, 41-44, 53-
642 55, 69, 74-81]. (7) The raw data has been deposited in the ProteomeXchange
643 Consortium: egg somatic proteome (PXD022516; [35]), secretome and somatic
644 proteomes of NEJ, immature and adult parasites (PXD003214 [41]; PXD007255 [11];
645 PXD021221 [12]; PXD011991 [43]); adult extracellular vesicles (PXD002570 [44];
646 PXD008737 [75]; PXD007782 [55]; PXD016561 [42]); tegument (PXD003911 [81];
647 PXD005099 [69]. Proteomic profiles of host responses to *F. gigantica* infections are
648 deposited in the iprox database (IPX0002287000; [82]) and ProteomeXchange
649 Consortium (PXD011576; [83]).

650 Post-translational modifications: Only a few studies have been carried out
651 investigating glycosylation and phosphorylation of *Fasciola* spp. proteins namely (9)
652 glycomics analyses of *F. hepatica* [32, 42, 55, 84, 85] and (10) phosphoproteomics
653 analysis of adult *F. gigantica* [86].

654 **Figure I (in Box 1). Schematic of available omics datasets for *Fasciola* spp.**
655 Figure created using Biorender; Parasite art by https://smart.servier.com.

656

657 **Box 2. Highly regulated multi-gene families**

658 Within the *Fasciola* spp. genomes, key gene families have expanded and display
659 diverse functionality, highlighting the critical roles these molecules play in parasite
660 biology and their interaction with their hosts [33]. These genes are strictly regulated
661 and are expressed at strategic points of the life cycle.

662

663 *Cathepsin cysteine peptidases*

664 The genes that comprise the large functionally diverse cathepsin cysteine peptidase
665 gene family (23 cathepsin L and 11 cathepsin B genes) play a critical role within both
666 the intermediate and definitive hosts, reflected by the high levels of stage-specific
667 transcription of cohorts of cathepsin peptidase genes [45]. Strikingly, only the
668 cathepsin L peptidases are abundantly transcribed by the snail associated stages [37].
669 In contrast, differential transcription of cathepsin B and L peptidase genes is observed
670 by the stages present within the mammalian host [37, 45]. These peptidases play
671 critical roles for metacercariae excystment, digestion of liver tissue and
672 macromolecules during migration, haemoglobin digestion during feeding and
673 immunomodulation by cleaving immunoglobulins [45].

674

675 *G protein-coupled receptors (GPCRs)*

676 The GPCR superfamily, comprised of five main families (GRAFS nomenclature),
677 regulate a range of physiological functions including neurotransmission, sensing the
678 environment, metabolism and cellular differentiation and growth [87]. To date, 147
679 GPCRs have been identified within the *F. hepatica* genome, with the rhodopsin family
680 highly represented (135 GPCRs), representing the largest number of GPCRs
681 described for a parasitic helminth [88]. Of the 77 GPCRs expressed by the life cycle
682 stages associated with the mammalian host, the majority (64) are abundantly
683 transcribed by the actively migrating NEJ stage implying an important role in migration,
684 growth and development of these liver-migrating stages [88].

685

686 *Tubulins*

687 Cytoskeletal microtubules are involved in a variety of cellular processes including
688 cellular structure and the separation of chromosomes during mitosis [89]. They are
689 also the targets of the benzimidazole class of anthelmintics [90]. In eukaryotes, the
690 genes encoding these microtubule proteins, α and β-tubulin, generally form a large
691 family, with distinct transcription encompassing cohorts of genes displaying
692 constitutive expression as well as genes with cell-specific transcription [91]. The
693 *Fasciola* spp. tubulin genes follow this pattern with constitutive transcription of five
694 tubulin genes implying a house-keeping role (α2, α3, β2, β3, β4) and the remaining
695 members of the gene family (α1, α4, α5, β1, β5, β6) displaying a dramatic upregulation
696 by the immature and adult flukes indicating a more specialised yet to be determined
697 role [10, 37].
698
699 **Figure legends**
700 **Figure 1. *Fasciola hepatica* life cycle.** (A) The liver fluke life cycle requires two hosts;
701 a snail intermediate host where asexual reproduction occurs over a 6-8-week period
702 and a mammalian definitive host where sexual reproduction occurs. Eggs are
703 produced by the adult parasites within the bile ducts, that are typically detected
704 approximately 10-12 weeks after ingestion of the infective metacercariae stage. A
705 summary of the key metabolic and developmental changes that occur across the life
706 cycle is included. (1) Metacercariae are ingested by mammalian host; (2)
707 Metacercariae excyst in small intestine releasing newly excysted juveniles (NEJ); (3)
708 Migration across the peritoneal cavity to the liver; (4) Migration through the liver
709 parenchyma to the bile ducts, where the mature adult parasites produce eggs that are
710 passed in the faeces; (5) Eggs hatch and release miracidia; (6) Miracidia penetrate
711 snail tissue; (7) Successive generation of sporocysts and rediae; (8) Cercariae

712 released from snail; encyst on vegetation as metacercariae. Figure created using
713 Biorender; Parasite medical art provided by Les Laboratories Servier,
714 https://smart.servier.com. (B) Graphical representation of the number of transcripts
715 from the comparable *F. hepatica* [10] and *F. gigantica* [37] stage-specific
716 transcriptome datasets generated by ggplot2 in R. The colours represent the number
717 of transcripts shared by all the life cycle stages (green), shared by at least two life
718 cycle stages (aqua/blue) and those unique to that specific dataset (purple).

719

Table 1. Efficacy of fasciolosis vaccines in large ruminants; comparison between single antigen vaccines versus multi-antigen cocktails.

Combination vaccine trials					
Trial	FhGST, FhTeg [59]	FhCL1, FhLAP [60]	FhTeg1^, FhTeg5^ [61]	FhCL1, FhHDM, FhLAP, FhPrx [58]	FhStf1, FhStf2, FhStf3, FhKT1 [62]
Form	Recombinant	Recombinant chimera	Recombinant	Recombinant	Recombinant
Adjuvant	FCA/FIA	Quil A	FCA/FIA	Montanide ISA 61 VG	Montanide ISA 61 VG
Host; No. animals/group	Cattle; 6	Sheep; 5	Cattle; 7	Sheep; 10	Sheep; 14
Vaccine efficacy	33%	46.5%	0%	37.2%	17.4%

Single antigen vaccine trials						
Antigen	**Form**	**Adjuvant**	**Host**	**No. animals /group**	**Vaccine efficacy**	**Reference / Reviewed by**
FhCL1*	Native	FCA/FIA	Cattle	4	52.5-69.5%	[92]
	Recombinant	Montanide ISA 206 VG	Cattle	13	49.2%	

FhGST	Native	FCA	Sheep	9	57%	[92]
	Native	Quil A+ 33% w/v Montanide 80	Cattle	8	69%	
	Native		Goat	6	0%	
	Recombinant	FCA/FIA Quil A	Goat	7	0%	
FhHDM	Native	Quil A	Sheep	5	15%	[6]
	Synthetic	Quil A	Sheep	5	6%	
FhKT1/KTM	Native	FCA/FIA	Sheep	Not stated	0%	[92]
	Native	Quil A	Cattle	Not stated	0%	
FhLAP	Native	FCA/FIA	Sheep	10	89.6%	[92]
	Recombinant	FCA/FIA	Sheep	10	83.8%	
	Recombinant	Adyuvac 50	Sheep	10	74.4%	
	Recombinant	Alum	Sheep	10	86.9%	
FhPrx	Recombinant	Quil A	Goat	7	33.04%	[92]
	Recombinant	Quil A	Goat	7	0%	

FhTeg	Recombinant	FCA/FIA	Cattle	6	0%	[59]

*Representative trials using native and recombinant forms

^ Single antigen vaccine trials not described for these antigens

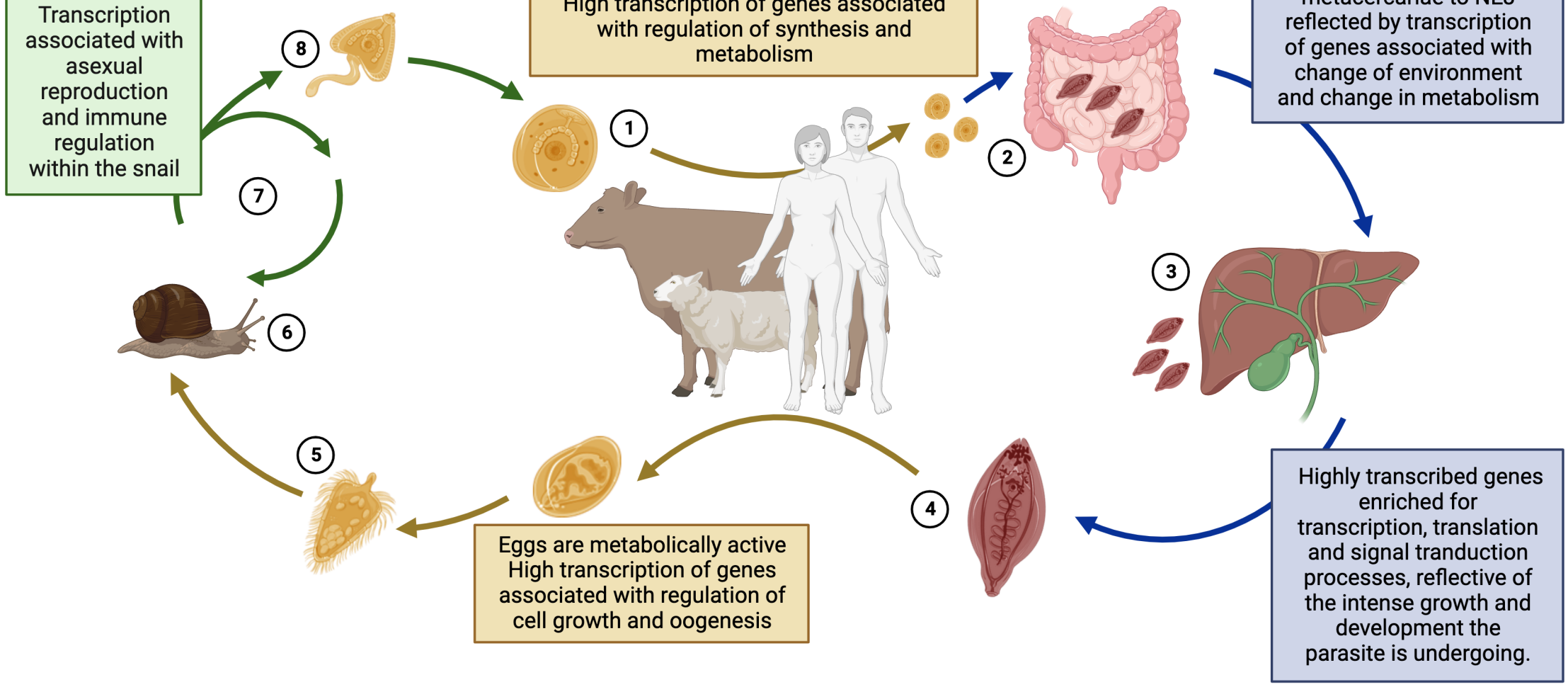
A

Cathepsin peptidases play an important role during the *Fasciola* spp. lifecycle.
Cathepsin B peptidases: abundantly transcribed by the cercariae stage through to the adult stage parasite.
Cathepsin L peptidases: abundantly transcribed by the rediae stage through to the adult stage parasite.

Transcription associated with asexual reproduction and immune regulation within the snail

Metacercariae are metabolically active. High transcription of genes associated with regulation of synthesis and metabolism

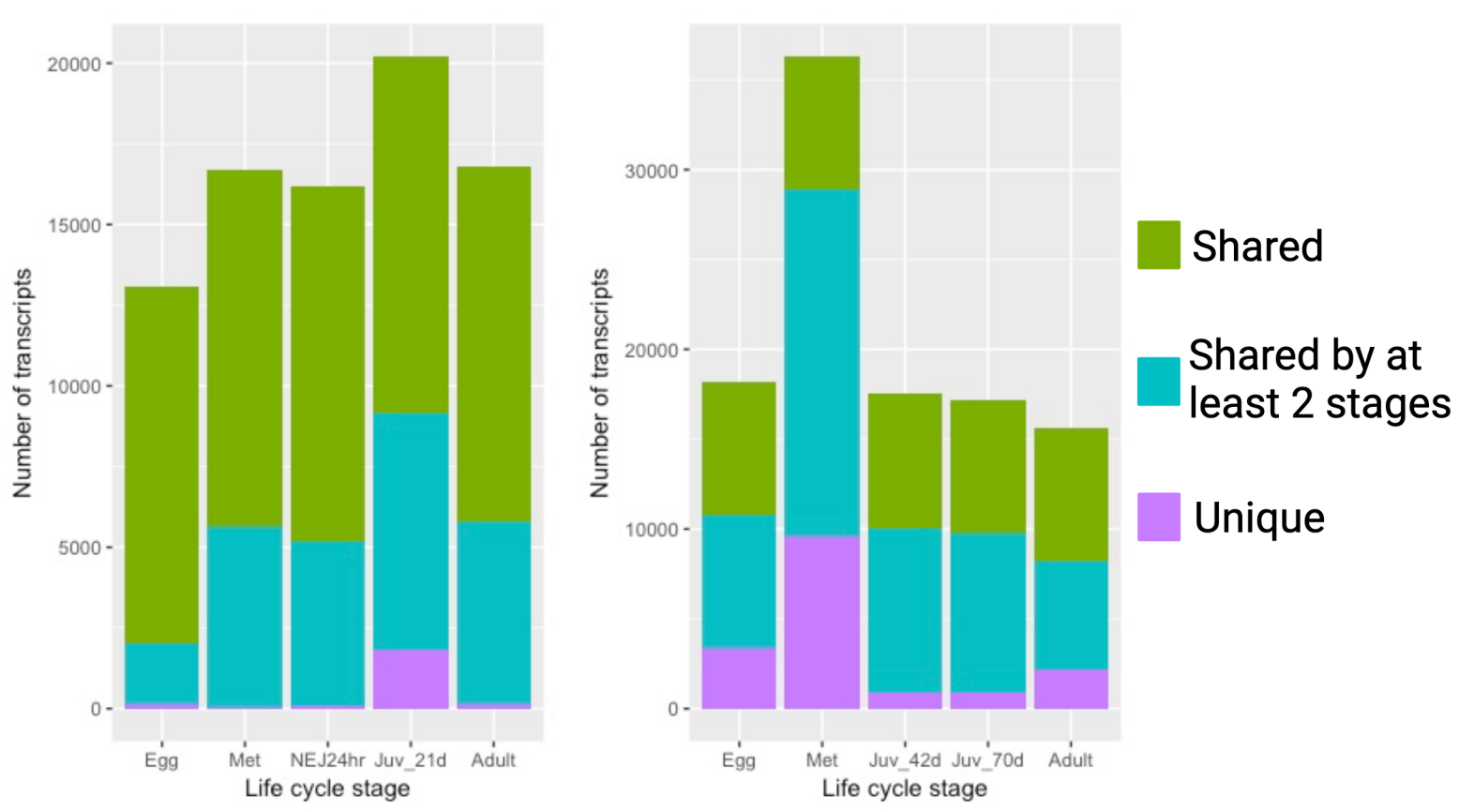
Transition from metacercariae to NEJ reflected by transcription of genes associated with change of environment and change in metabolism

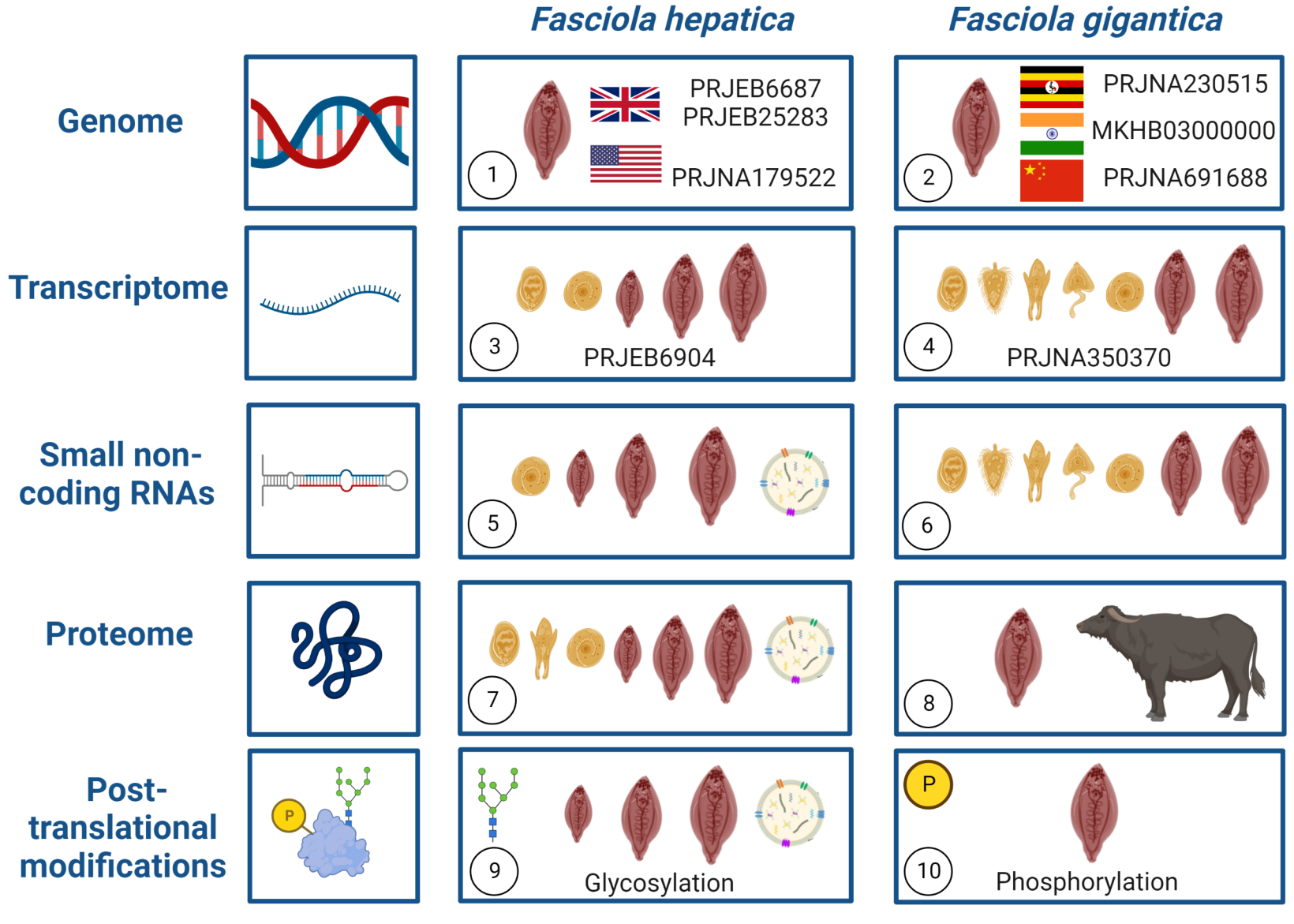


Eggs are metabolically active
High transcription of genes associated with regulation of cell growth and oogenesis

Highly transcribed genes enriched for transcription, translation and signal tranduction processes, reflective of the intense growth and development the parasite is undergoing.

B




Fasciola hepatica
Fasciola gigantica
Genome
PRJEB6687
PRJEB25283
PRJNA179522
1
PRJNA230515
MKHB03000000
PRJNA691688
2
Transcriptome
3
PRJEB6904
4
PRJNA350370
Small non-coding RNAs
5
6
Proteome
7
8
Post-translational modifications
P
9
Glycosylation
P
10
Phosphorylation