Heterogeneous glycosylation of proteins from *Fasciola hepatica* invasive stage reveals higher complexity in parasite-host interactions

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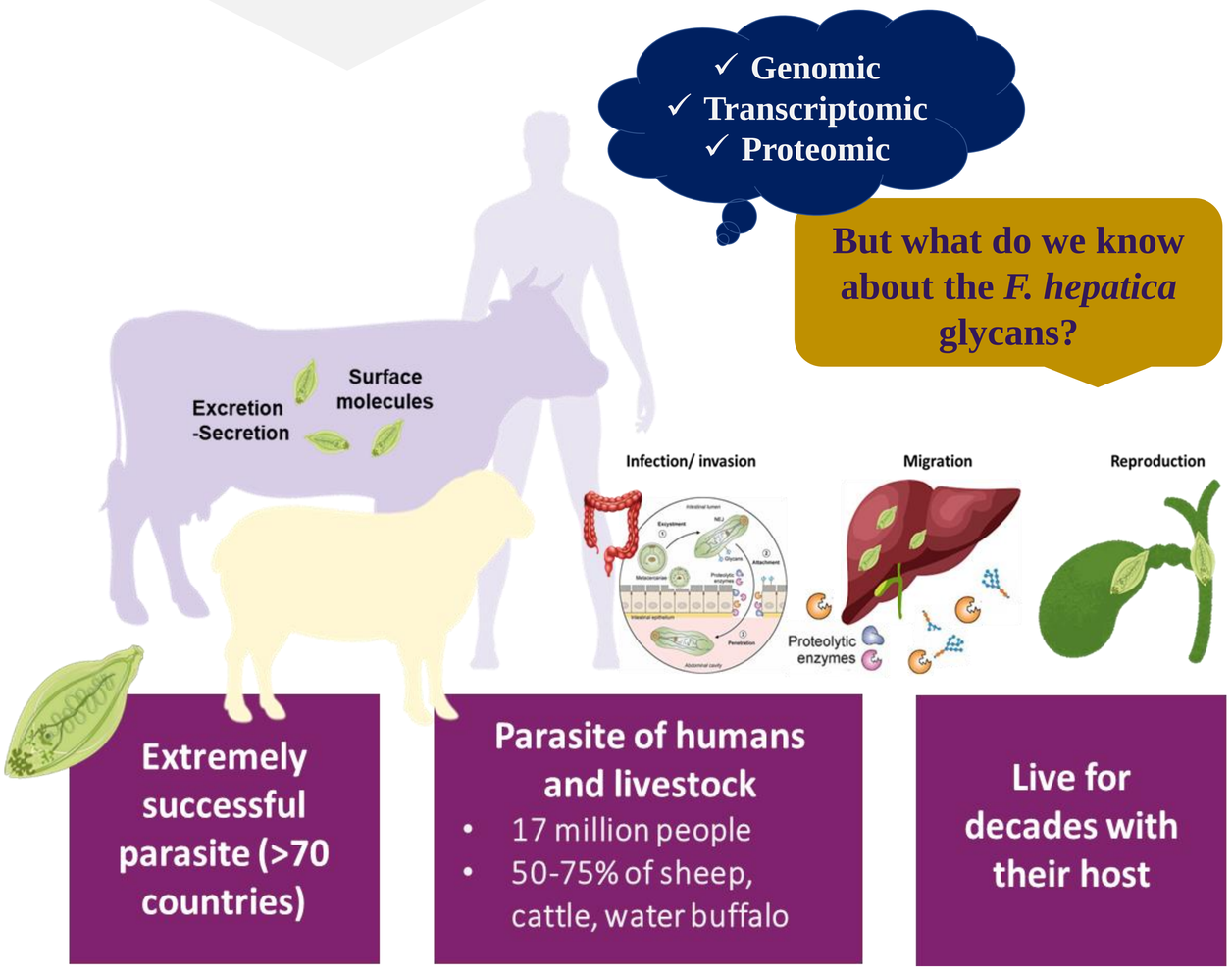
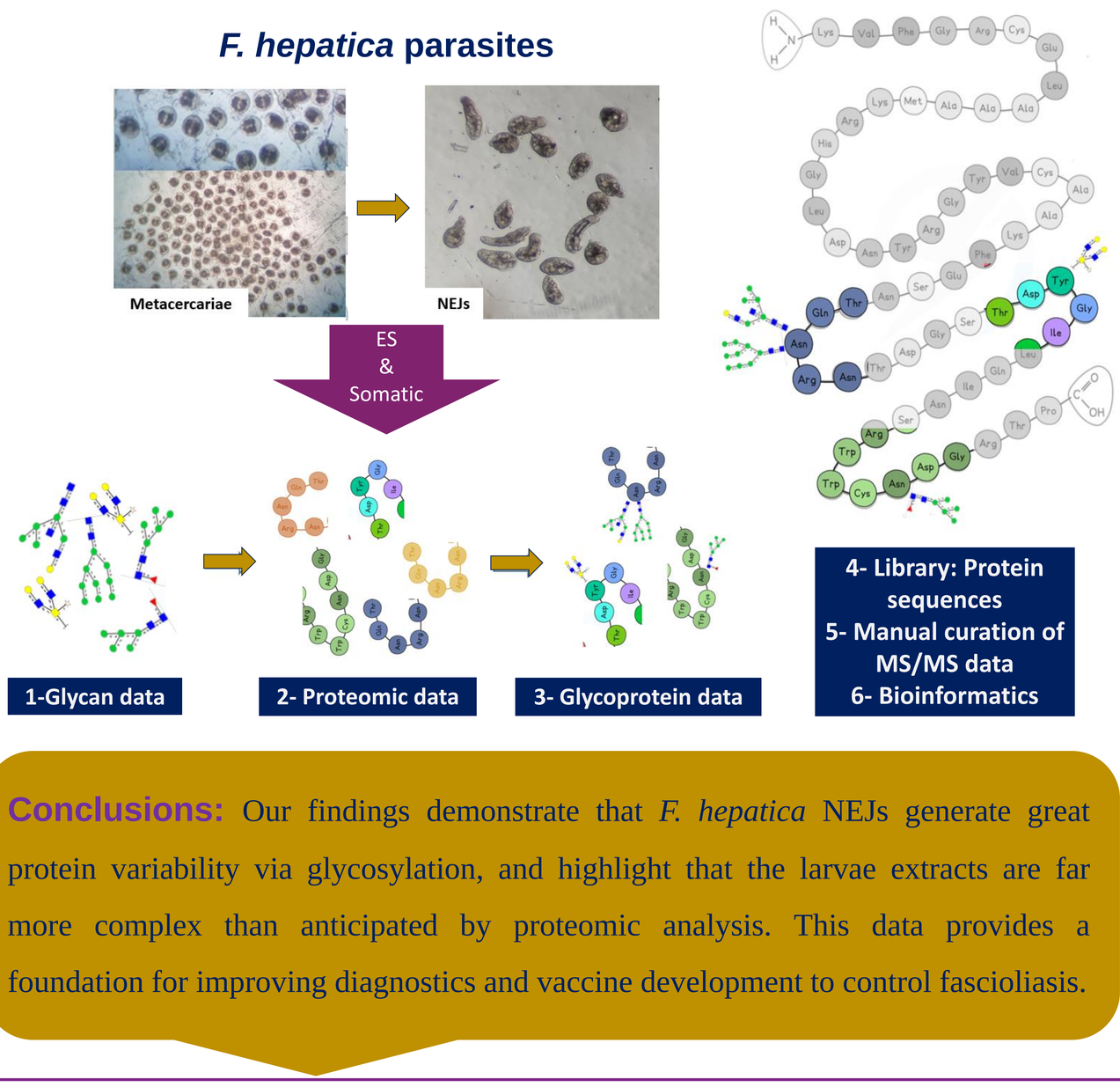
Background and aim: *Fasciola hepatica* is a parasitic trematode that uses glycosylated excreted-secreted (ES) and surface molecules to interact with host cells and tissues, and to evade damage caused by cellular and immune responses during host invasion. Despite the unknown glycosylation state of many of the ~100 different proteins found in the ES of the immature invasive stage of *F. hepatica* (NEJs), several are extensively used as diagnostic and vaccine targets. To develop more effective strategies against fascioliasis, information on the glycosylation profile of individual NEJs proteins is critical. **In this study, we used a combination of glycan, glycopeptide, and proteomic analyses, along with bioinformatics tools, to identify the glycosylation status of individual *F. hepatica* NEJs proteins.**

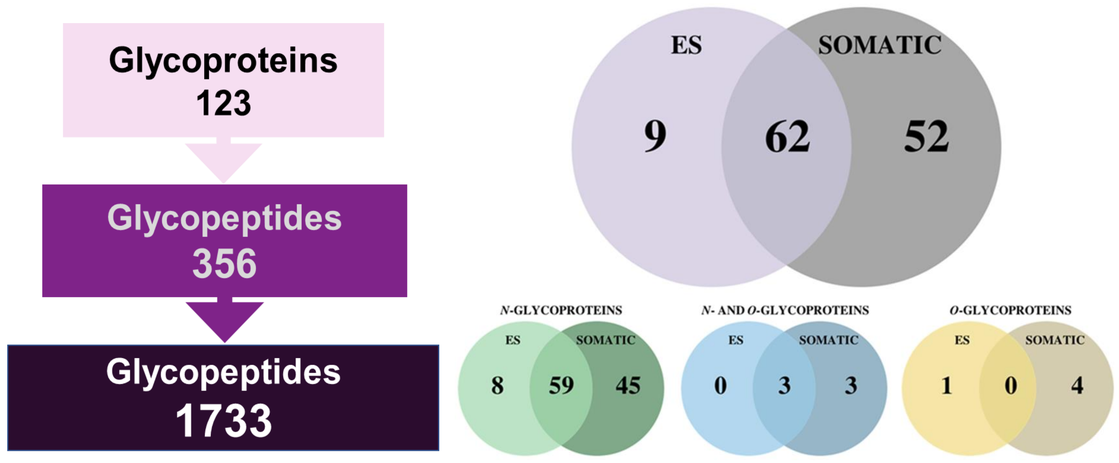


Material and Methods



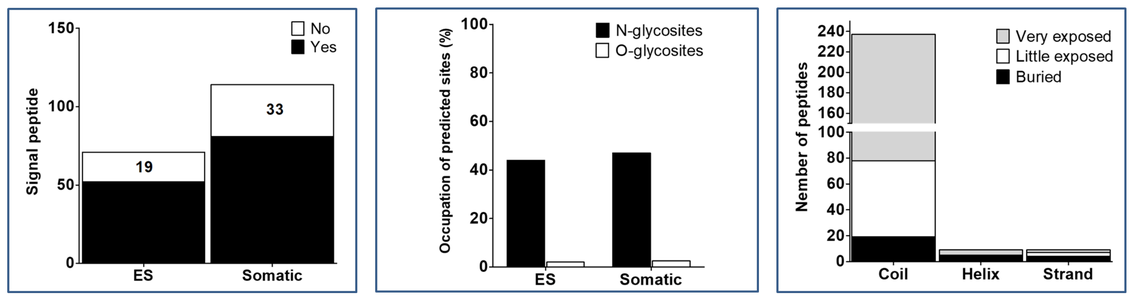
Results

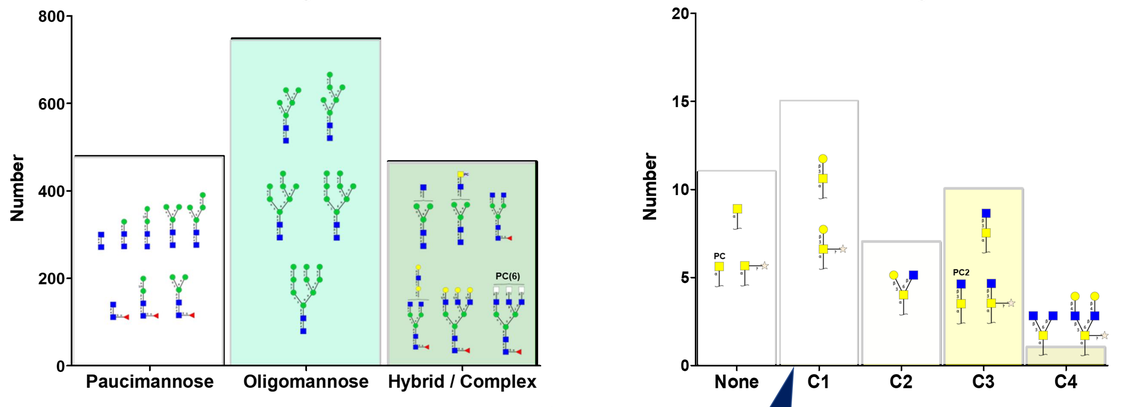
Unique glycan motifs, such as PC and multi-PC terminals, and xylosylated O-glycan cores, were found in 25 distinct NEJs glycoproteins, including cathepsin peptidases B and L, which are well-known vaccine and diagnostic targets. Furthermore, many parasite proteins carried highly truncated N-glycans and structures with undefined linkages that could not be assigned (i.e., HexNAc2Hex4dHex1), and the roles of which in parasite infection are largely unknown. These structures modify glycoproteins that are excreted-secreted or predicted to be membrane-bound, suggesting that they play key roles in NEJs interactions that command host invasion.

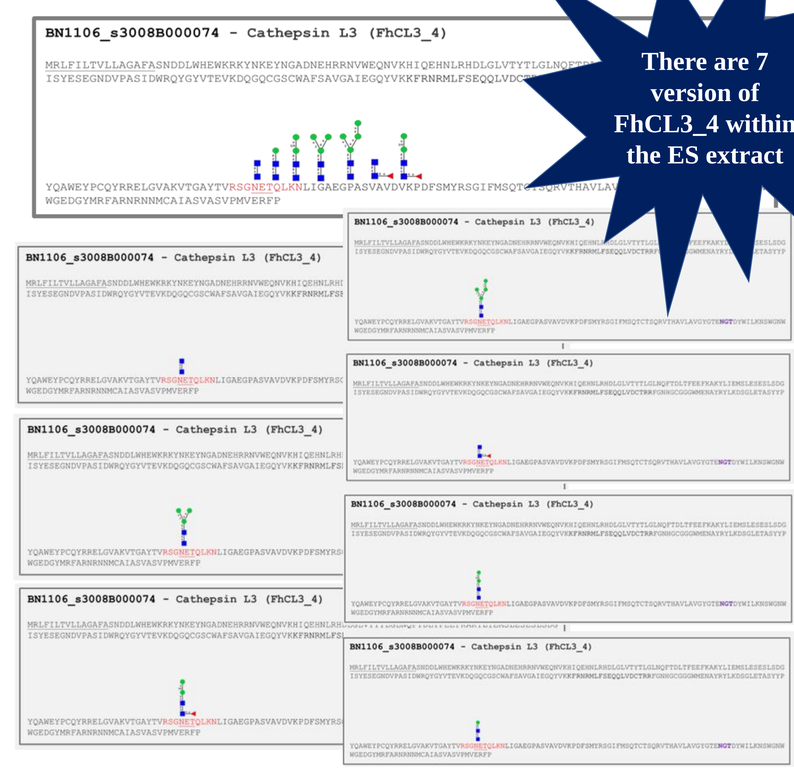


***F. hepatica* uses glycans to generate protein diversity.**

Most glycoproteins are present in both ES and Somatic NEJs extract!







123 glycoproteins characterized

71 glycoproteins are **excreted-secreted** by NEJs

56 *N*-glycans
16 *O*-glycans
are used to modify the NEJs glycoproteins

There is major glycan heterogeneity within NEJs glycoproteins

3.0 Glycopeptides Protein

4.6 Glycan forms Glycopeptide

14 Glycan forms Protein

Conclusions: Our findings demonstrate that *F. hepatica* NEJs generate great protein variability via glycosylation, and highlight that the larvae extracts are far more complex than anticipated by proteomic analysis. This data provides a foundation for improving diagnostics and vaccine development to control fascioliasis.

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