Characterisation of protective vaccine antigens from the thiol-containing components of the excretory/secretory material of *Ostertagia ostertagi*

Daniel R. G. Price[1*], Phil Steele[1], Kevin McLean[1], Dorota Androscuk [1], Alasdair J. Nisbet[1], Tom N. McNeilly[1]

[1]Moredun Research Institute, Pentlands Science Park, Edinburgh EH26 0PZ, United Kingdom.

[*]Email: dan.price@moredun.ac.uk

Introduction

The gastrointestinal nematode *Ostertagia ostertagi* causes serious health and welfare issues as well as substantial economic losses in cattle. Control of this parasite is reliant on the use of anthelmintics, but the widespread and prolonged use of these drugs has resulted in the selection of resistant worms. Alternative control approaches, such as vaccination, are needed to provide robust and sustainable control of these parasites.

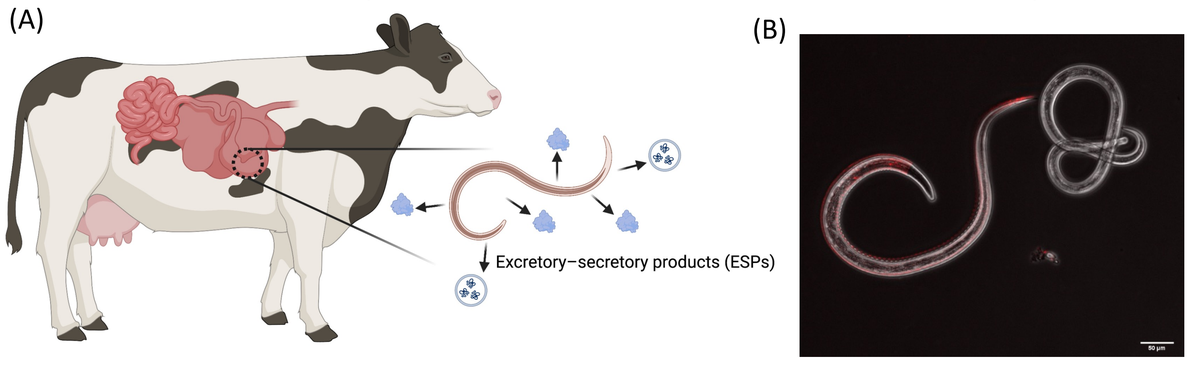


Figure 1. (A) *Ostertagia ostertagi* develop in the gastric stomach of cattle and L4 and adult parasites produce excretory-secretory products (ESPs) that are essential for establishment and maintenance of an infection. (B) Image of adult female *O. ostertagi*. Scale bar 50 µm (image courtesy of Marc Faber, MRI).

Preparation of *O. ostertagi* ES-thiol proteins

Adult *O. ostertagi* nematodes were collected at post-mortem from infected cattle and cultured *in vitro* for up to 96 h. ESPs were collected from the culture supernatants every 24 h and thiol-containing proteins purified by affinity chromatography using thiol-Sepharose.

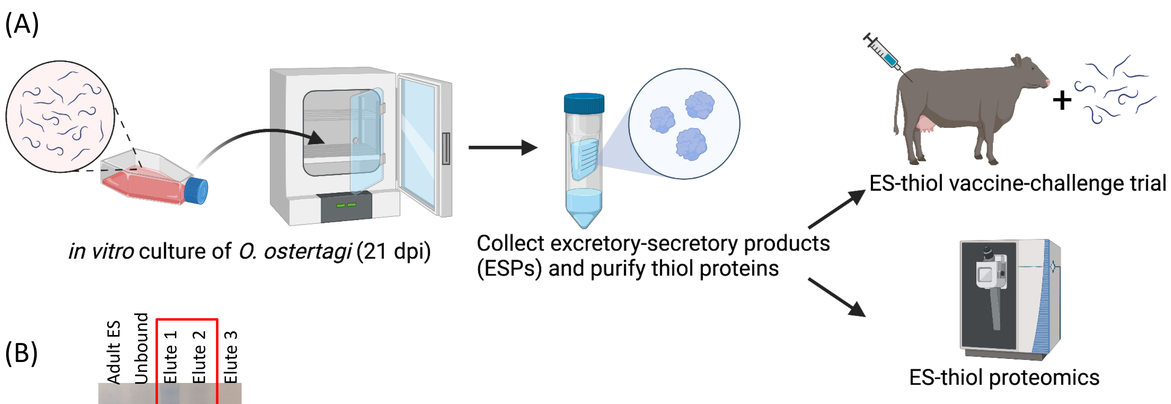


Figure 2. (A) Preparation and characterisation of *O. ostertagi* (adult) ES-thiol proteins. ES-thiol proteins were used in vaccination trials and for proteomic characterisation. (B) SDS-PAGE analysis of *O. ostertagi* ESPs and fractions from ES-thiol affinity purification. ES-thiol fractions elute 1 and 2 were used for vaccine-challenge trials and proteomic analysis.

Vaccine – challenge trial with *O. ostertagi* ES-thiol

Cattle were vaccinated three times with *O. ostertagi* ES-thiol + Quil A and control animals received Quil A adjuvant only (each group n = 7). After the final vaccination each animal was challenged three times per week with 5,000 L3 *O. ostertagi* for eight weeks. To monitor the infection faecal worm egg counts (FWECs) were preformed on each animal three times per week for the duration of the infection.

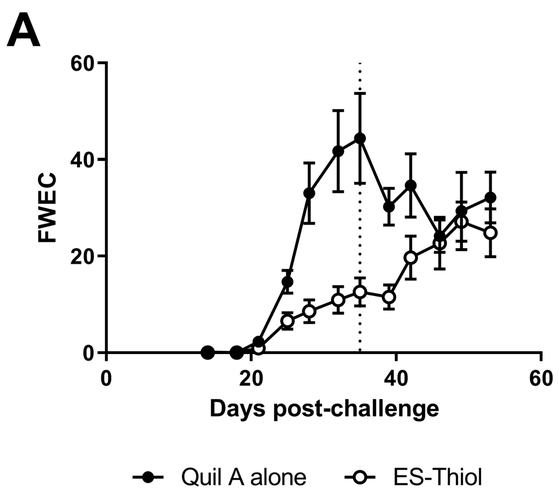


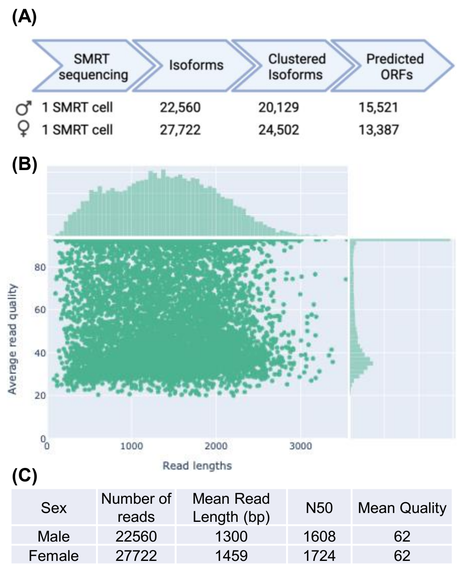
Figure 3. Effects of immunization of cattle with *O. ostertagi* ES-thiol on (A) daily mean faecal worm egg counts (FWEC) and (B) cumulative FEWC (cFWEC) (B) after challenge infection. Each data-point represents mean FWEC +/- SEM, n=10. *** = $p < 0.001$ (Mann-Whitney Test)

Outcome:

- FWEC in ES-thiol vaccinated animals is reduced by 60% relative to control (adjuvant only) vaccinated animals.

Generation of *O. ostertagi* sequence database

O. ostertagi ES-thiol proteins (Figure 2B, lanes Elute 1 and 2) were analysed by high-resolution proteomics using a Q Exactive™ Plus Obritrap mass spectrometer (Thermo Fisher Scientific). To analyse the proteomic spectra data we used Pac-Bio isoform sequencing (Iso-Seq) to generate a full-length transcript database.



Sex	Number of reads	Mean Read Length (bp)	N50	Mean Quality
Male	22560	1300	1608	62
Female	27722	1459	1724	62

Figure 4. Summary of PacBio Iso-Seq sequencing for adult *O. ostertagi* males and females. (A) Bioinformatic pipeline and number of full-length isoforms generated for *O. ostertagi* male and female parasites. (B) Histogram showing read length (bp, x-axis) against read quality (Phred score, y-axis) for all male and female isoforms (50,282 isoforms in total). (C) Sequencing summary statistics for *O. ostertagi* male and female Iso-Seq libraries.

Classification of identified *O. ostertagi* ES-thiol proteins

Proteomic analysis of *O. ostertagi* ES-thiol resulted in the identification of 490 proteins. The identified proteins were classified using GO analysis and Interpro domain classification.

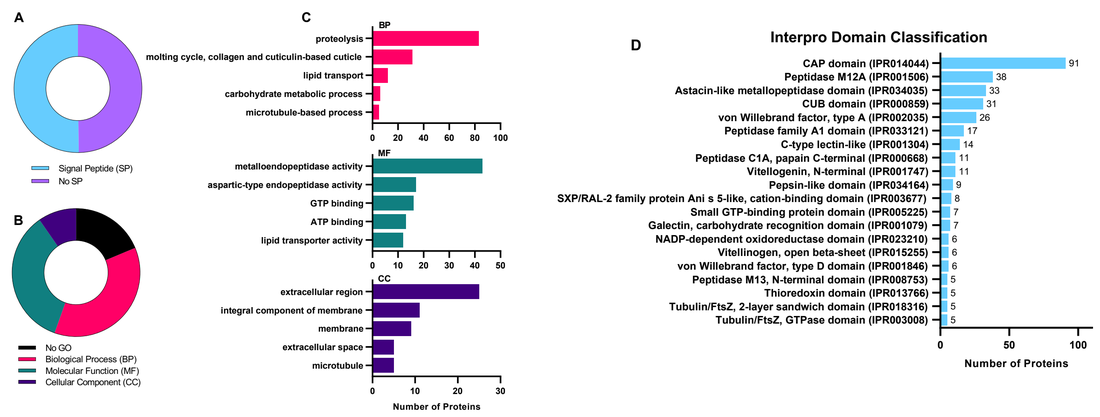


Figure 5. GO classification and Interpro domain classification of identified *O. ostertagi* ES proteins. (A) Proportion of ES proteins containing a secretion signal (n=490). (B) GO distribution of identified ES proteins. Proteins are classified into three broad GO categories: biological process (red); molecular function (green) and cellular component (purple). (C) Direct GO count showing the top five GO terms for each category. Some proteins are included in more than one category. (D) Interpro domain classification of identified *O. ostertagi* ES proteins showing top twenty categories. Some proteins are included in more than one category.

Outcome:

- The most numerous ES-thiol proteins, with 91 proteins identified, contain a CAP domain and belong to the sperm-coating protein/Tpx/antigen 5/pathogenesis-related protein 1 (SCP/TAPS) family.
- Proteinases, such as the astacin-like metalloproteinases, are abundant in O. ostertagi ES-thiol proteins.

High Density Peptide arrays to identify immunogenic B-cell epitopes

In conjunction with PEPperPRINT GmbH (Heidelberg, Germany) we have produced a high density peptide array of the 490 identified *O. ostertagi* ES-thiol proteins. The array includes 15-mer peptides with an overlap of 10 or 11 amino acids. Each peptide is printed in duplicate, giving a total of 68,280 printed spots.

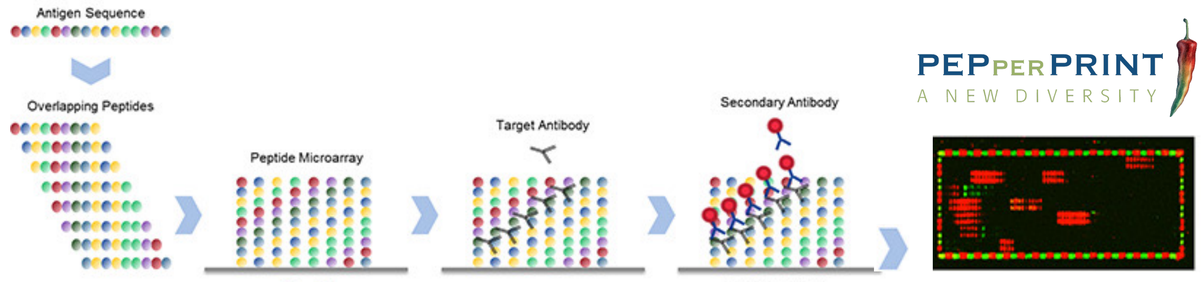


Figure 6. Overview of the PEPperPRINT peptide array technology. Peptides (15-mers) are printed onto a glass slide using a laser printer. Each peptide overlaps by 10 or 11 amino acids and covers each of the 490 *O. ostertagi* ES-thiol proteins.

Outcome:

- Peptide arrays have been produced and screened with pre- and post- vaccination sera. We are currently analysing data to identify immunogenic epitopes.
- Identified antigens will inform future *O. ostertagi* recombinant vaccine design.

Acknowledgements

The work was supported in part by a Moredun Foundation Research fellowship awarded to DRGP. All authors receive funding from the Scottish Government Rural and Environment Science and Analytical Services Division (RESAS) and this work was partly funded from that source.

RESAS Rural & Environmental Science and Analytical Services

SEFARI

Scottish Government Riaghaltas na h-Alba gov.scot

Moredun Foundation