Thesis Abstract & Published Papers

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Project Title:	**PhD: Anthelmintic control in sheep: Proteomic fingerprinting to identify markers for monitoring anthelmintic resistance in *Haemonchus contortus***		
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Abstract:

Gastro-intestinal parasitic nematode infections are a major health and economic problem in the sheep farming industry worldwide. The current use of chemical anthelmintics has led to the resistance of the parasites in the field and a reliable method to monitor it is required to maintain their efficiency. In this study, we investigated the resistance mechanisms of *Haemonchus contortus* to ivermectin, using bioinformatic, proteomic and transcriptomic techniques, in order to provide new biomarkers of resistance.

A putative protein database was produced from publicly available *H contortus* Expressed Sequence Tags, using the bioinformatic software, prot4EST. The usefulness of this database was then tested by building a cytosolic two-dimensional electrophoresis proteome profile of proteins. 62 of the 100 highest expressed protein spots were identified by Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight Mass Spectrometry followed by Peptide Mass Fingerprint search against the newly-constructed Expressed Sequence Tags protein database. This method was finally validated by performing tandem mass spectrometry and Ion search analysis of a selection of 20 of these proteins.

The cytosolic proteomes of two ivermectin-resistant and one ivermectin-susceptible *H contortus* isolates were compared by Difference Gel Electrophoresis after 1 hour *in vitro* culture containing ivermectin. Statistically significant 1.5-fold up- or down-regulated proteins were identified by tandem mass spectrometry followed by Ion searches against the Expressed Sequence Tags protein database. This study revealed the up-regulation of Globin-1 and several enzymes involved in energy metabolism in the resistant isolates. It also demonstrated the down-regulation of Actin 3, the up-regulation of Unc-60 (an actin depolymerising factor), the down-regulation of LEV-1 1 (a tropomyosin which inhibits Unc-60) and Heat Shock Protein-1 (molecular chaperone), all required for correct muscle contraction. Finally, this study highlighted the up-regulation of two glutathione transferases, involved in xenobiotic detoxification. The study of messenger ribonucleic acid levels by quantitative Real Time PCR of Actin-3, UNC-60, Globin-1, LEV-11, Heat Shock Protein 1 and the two Glutathione transferases did not show differences in expression between the resistant and susceptible isolates.

After glutathione affinity chromatography, glutathione transferase sub-proteomes were compared by two-dimensional electrophoresis between resistant and susceptible isolates subjected to two different conditions: (i) after 1 how *in vitro* culture not containing ivermectin, and (ii) after 3 hour *in vitro* ivermectin exposure. This comparison demonstrated the plasticity of the glutathione

transferase sub-proteome and higher glutathione transferase specific activity in the resistant isolate. Glutathione transferase protein spot identifications by tandem mass spectrometry followed by Ion searches and phylogenetic classification, resulted in identification of three Sigma class glutathione transferases. HCC00159, matched to *H contortus* HcGST-1, was shown to be up-regulated in ivermectin-exposed resistant isolates.

These different biomarker candidates provide new leads to understand the resistance mechanism to ivermectin, involving muscle contraction, xenobiotic detoxification and, maybe, energy metabolism. Thus, reliable resistance monitoring methods may require simultaneous quantification of numerous components in the cell.

Published Papers:

1. **Paul Millares** et al, **The soluble proteome phenotypes of ivermectin resistant and ivermectin susceptible Haemonchus contortus females compared.** Veterinary Parasitology vol 190 Iss 1-2 Nov 2012 p104-113. http://dx.doi.org/10.1016/j.vetpar.2012.06.009
2. **Paul Millares** et al, **Proteiomic profiling and protein identification by MALDI-TOF mass spectrometry in unsequenced parasitic nematodes.** PLOS ONE 7(3):e33590 http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0033590