Thesis Abstract & Published Papers

Name:	**Alison Dicker**		
Project Title:	**PhD: Anthelmintic control in sheep: Comparative gene expression studies of anthelmintic resistance in the parasitic nematode *Teladorsagia circumcincta***		
Project No:	**7382 (3 PhD's)**	Date:	**Oct 2006 - 2010**

Abstract:

Anthelmintic resistance in parasitic nematodes of small ruminants is widespread and, in some parts of the world, threatens the sustainability of sheep production. The mechanisms whereby parasitic nematodes become resistant to anthelmintics, particularly ivermectin, remain to be determined. The majority of studies to date have investigated target site mutations; relatively little attention has been paid to the role of gene expression changes. The present study focused on *Teladorsagia circumcincta;* the predominant parasitic gastrointestinal nematode species in the UK and the predominant resistant species. The role of changes in gene expression were investigated in an ivermectin-susceptible isolate (CVL) and a multidrug resistant isolate (MOTRI), utilising a range of molecular biological techniques.

In the first experiment, a panel of novel putative ivermectin resistance genes were identified from *T: circumcincta,* comprising 11 partial P-glycoprotein (Pgp) and 3 partial Cytochrome P450 (CYP) sequences. Both Pgps and CYPs have been implicated in the handling and metabolism of xenobiotics in other biological systems, but have not been investigated in *T: circumcincta* to date. Initial results, using semi-quantitative PCR identified changes in expression of this panel of genes between the CVL and MOTRI isolates.

Constitutive differences in expression of the Pgps and CYPs between CVL and MOTRI were determined using the ΔΔCt TaqMan® real-time PCR method. A statistically significant increase in expression was observed for *TeciPgp-9* NBD2 across all life-cycle stages but most notably in eggs (55-fold increase). A statistically significant reduction in expression of *TeciPgp-2* NBD2 was observed in all but the adult stages of MOTRI compared to CVL. Analysis of a 208 base pair sequence of *TeciPgp-9* NBD2 identified high levels of polymorphism, with at least four non-coding SNPs evident in the MOTRI isolate. These results merit further investigation.

Inducible changes in the expression of the Pgps and CYPs were investigated in MOTRI before and after ivermectin treatment, using real-time PCR. Statistically significant fold changes in expression in most of the genes occurred in at least one lifecycle stage. Inducible expression of *TeciPgp-2* NBD2 and *TeciPgp-9* NBD2 was investigated further by comparing adult MOTRI parasites with those recovered three days after *in vivo* ivermectin exposure, and by exposing pools of MOTRI xL_3 to ivermectin in the larval migration inhibition test. The survivors of ivermectin exposure exhibited a statistically significant reduced 13.68-fold expression of *TeciPgp-2* NBD2 compared to MOTRI. Similarly, the MOTRI xL3 able to migrate in the presence of ivermectin in the LMIT had a 1.88-fold reduction in *TeciPgp-2* NBD2 expression compared to MOTRI xL3 unexposed to ivermectin.

These results indicate that inducible changes in *TeciPgp-2* NBD2 and *TeciPgp-9* NBD2 expression can occur, but the experimental design is critical to being able to identify the changes.

In a more global approach, the transcriptomic response of MOTRI adults to *in vitro* ivermectin exposure was investigated using Roche 454 sequencing, generating 98,685 novel EST sequences, providing an important resource for a genome resource-poor organism. Objective bioinformatic analysis of the two datasets revealed statistically significant differences in the mean expression levels of the KEGG orthologous groups for 'translation', 'amino acid metabolism' 'carbohydrate metabolism' and 'xenobiotic degradation and metabolism'. On combining the two datasets, and through application of a novel statistical method, 16 clusters of ESTs were identified as containing statistically significant differences in the mean proportion of exposed reads compared to unexposed reads under the conservative model, whilst a further 355 clusters were found to have statistically significant differences under the liberal model.

One-way suppression subtractive hybridisation (SSH) was used to identify genes exhibiting increased expression in MOTRI adults compared to CVL adults. 28 contiguous sequences were identified from the SSH experiment; 6 contiguous sequences were selected for validation; 5 of these results were confirmed using semi-quantitative PCR. Each contig was BLAST searched against the Roche 454 dataset; contig SSH14 aligned most closely to one of the statistically significant clusters in the conservative model, SSHs 5, 6, 10 and 23 aligned most closely to statistically significant clusters in the liberal model. This suggests that changes in expression in these sequences occur both constitutively, between CVL and MOTRI isolates, and inducibly, following ivermectin exposure.

This work has shown that changes in gene expression, particularly the constitutively reduced expression in *TeciPgp-2 NBD2* and the constitutively increased expression in *TeciPgp-9 NBD2* (coupled with the presence of SNPs) could play a role in allowing multidrug resistant *T. circumcincta* to survive ivermectin exposure. Roche 454 sequencing and SSH approaches identified gene expression changes associated with *in vitro* ivermectin exposure and ivermectin resistance. These could form the basis of a novel panel of candidate resistance genes whose altered expression profiles may allow multidrug resistant *T: circumcincta* to survive ivermectin exposure by some, as yet identified, mechanism. Finally, we have also shown that a multidrug *T. circumcincta* isolate is affected by ivermectin exposure and that changes in gene expression could have a role to play in the ivermectin resistance phenotype in *T: circumcincta.* The genetic changes underpinning these changes in gene expression remain to be elucidated, and need to be investigated in other isolates. These changes could form the basis of an ivermectin resistance molecular marker, to monitor the spread of resistance, and to evaluate management practices aimed at delaying its spread.

Published Papers:

1. **Alison J Dicker** et al, **Gene expression changes in a P-glycoprotein (Tci-pgp-9) putatively associated with ivermectin resistance in Teladorsagia cercumcincta**. International journal for parasitology vol 41 iss 9 Aug 2011 p935-942. http://dx.doi.org/10.1016/j.ijpara.2011.03.015

2. **Alison J Dicker** et al, **Teladorsagia circumcinta: The transcriptomic response of a multi-drug resistant isolate to ivermectin exposure in vitro**. Experimental parasitology vol 127 iss 2 Feb 2011 p351-356. http://dx.doi.org/10.1016/j.exppara.2010.08.019