

ABC TRANSPORT PROTEINS & DRUG RESISTANCE IN NEMATODES

Catherine E. James

PhD

2009



CERTIFICATE OF AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Production Note:
Signature removed prior to publication.

Catherine James

ACKNOWLEDGEMENTS

Without a doubt my biggest thank you goes to my supervisor, Assoc. Prof. Mary Davey. Thank you for all your guidance, support, patience and friendship. Because of your energy and enthusiasm I have had many wonderful experiences and opportunities during my PhD. I have appreciated every single one. Thank you.

I would like to acknowledge the students and staff in IBID - thank you for making it a pleasure to come to work every day. In particular a big thank you to my fellow '*team meat*' members Amanda Hudson and Irene Sotirchos. Thank you for the friendship, guidance and all the fun times.

I would like to thank Prof. Roger Prichard and his laboratory team, particularly Kathy Keller for the time spent in their laboratory. It was a truly special experience. A big thank you to the Australian Society for Parasitology and the ARC & NHMRC Network for Parasitology for funding my researcher exchange to McGill University, Montreal.

To my family and friends, particularly Mum, Dad, Robyn, Peta and Natalie- thank you for all your love and support, I am lucky to have you all in my life. To my partner Rohan, thank you for being my best friend, my rock and for all the love, support and patience during this experience. We did it!

Finally I would also like to acknowledge funding for the research conducted pursuant to this thesis was provided by Australian woolgrowers and the Australian Government through Australian Wool Innovation Limited.

TABLE OF CONTENTS

| | |
|-----------------------------|------|
| ABSTRACT | viii |
| JOURNAL PUBLICATIONS | x |
| PRESENTATIONS | x |
| LIST OF ABBREVIATIONS | xi |
| LIST OF FIGURES | xiv |
| LIST OF TABLES | xivi |

Chapter 1: Introduction 1

| | | |
|-------|--|----|
| 1.1 | Introduction | 2 |
| 1.2 | <i>H. contortus</i> | 2 |
| 1.3 | Control of <i>H. contortus</i> | 4 |
| 1.3.1 | Macrocyclic lactones | 4 |
| 1.3.2 | Benzimidazoles | 5 |
| 1.3.3 | Imidazothiazoles | 5 |
| 1.4 | Resistance to chemotherapy | 6 |
| 1.5 | Mechanisms of cellular resistance | 8 |
| 1.5.1 | Genetic changes in the drug target | 8 |
| 1.5.2 | Drug metabolism | 10 |
| 1.5.3 | ABC transport proteins | 12 |
| 1.6 | ABC transport proteins in parasites: the potential for MDR | 17 |
| 1.6.1 | ABC transport proteins in protozoan parasites | 17 |
| 1.6.2 | ABC transport proteins in metazoan parasites | 20 |
| 1.7 | <i>Caenorhabditis elegans</i> | 23 |
| 1.7.1 | A model nematode | 24 |
| 1.8 | Thesis objectives | 28 |

Chapter 2: Materials & methods..... 30

| | | |
|-------|---|----|
| 2.1 | Reagents | 31 |
| 2.1.1 | Chemicals | 31 |
| 2.1.2 | Biological reagents | 32 |
| 2.1.3 | Miscellaneous | 32 |
| 2.1.4 | Bacterial strains | 33 |
| 2.1.5 | <i>Caenorhabditis elegans</i> strains | 33 |
| 2.1.6 | <i>Haemonchus contortus</i> strains | 33 |
| 2.1.7 | Mammalian cell lines | 33 |
| 2.1.8 | Cloning vectors | 33 |
| 2.1.9 | Solutions | 33 |
| 2.2 | RNA methods | 34 |
| 2.2.1 | Dynal bead mRNA extraction | 34 |
| 2.2.2 | TRIzol RNA extraction | 35 |

| | | |
|---------|---|----|
| 2.2.3 | Estimating the concentration of nucleic acids..... | 35 |
| 2.2.4 | Determination of RNA quality | 35 |
| 2.2.5 | DNase I treatment | 35 |
| 2.2.6 | Reverse transcriptase PCR (RT-PCR) using oligo d(T) | 35 |
| 2.3 | DNA methods | 36 |
| 2.3.1 | Conventional PCR | 36 |
| 2.3.2 | Touchdown PCR..... | 36 |
| 2.3.3 | Random amplification cDNA ends (RACE) PCR..... | 36 |
| 2.3.4 | DNA sequencing..... | 36 |
| 2.3.5 | DNA sequence analyses..... | 36 |
| 2.4 | GENE cloning | 37 |
| 2.4.1 | Nucleic acid electrophoresis..... | 37 |
| 2.4.2 | DNA gel extraction..... | 37 |
| 2.4.3 | Restriction digestions | 37 |
| 2.4.4 | Ethanol precipitation of nucleic acids | 37 |
| 2.4.5 | Ligation reactions | 37 |
| 2.4.6 | Preparation of competent cells | 38 |
| 2.4.7 | Transformation of competent cells | 38 |
| 2.4.8 | Colony PCR..... | 38 |
| 2.4.9 | Plasmid preparations..... | 38 |
| 2.4.10 | Glycerol stocks..... | 39 |
| 2.5 | Real-time PCR..... | 39 |
| 2.5.1 | Real-time PCR reaction conditions | 39 |
| 2.5.2 | Primer Efficiency Analysis (verification of $\Delta\Delta C_T$ method by Livak and Schmittgen (2001)..... | 39 |
| 2.5.3 | Analysis of real time PCR data using the $\Delta\Delta C_T$ method | 39 |
| 2.5.4 | Primer Efficiency Analysis: Pfaffl (2001) method | 40 |
| 2.6 | Enzyme assays..... | 40 |
| 2.6.1 | Total intracellular glutathione/glutathione disulfide | 40 |
| 2.7 | <i>C. elegans</i> maintenance | 41 |
| 2.7.1 | Solid NGM worm culture | 41 |
| 2.7.2 | Harvesting <i>C. elegans</i> cultures..... | 41 |
| 2.7.3 | Worm glycerol stocks | 41 |
| 2.7.4 | Obtaining synchronous <i>C. elegans</i> cultures..... | 41 |
| 2.7.5 | RNA interference (RNAi)..... | 42 |
| 2.7.5.1 | RNAi controls | 42 |
| 2.7.5.2 | RNAi | 44 |
| 2.7.6 | MTT assay | 44 |
| 2.7.7 | Worm cytotoxicity assay..... | 44 |
| 2.7.8 | Response to drug treatment | 45 |
| 2.7.9 | Worm velocity | 45 |
| 2.8 | Mammalian cell culture methods | 45 |
| 2.8.1 | Mammalian cell culture | 45 |
| 2.8.2 | MTT cytotoxicity assay..... | 45 |
| 2.8.3 | Flow cytometry | 46 |
| 2.9 | Statistical analyses | 47 |
| 2.9.1 | Student's T-test..... | 47 |

| | |
|---|-----------|
| Chapter 3: A rapid colorimetric assay for the quantitation of the viability of free-living larvae of nematodes <i>in vitro</i> | 48 |
| 3.1 Optimisation of the MTT assay | 50 |
| 3.1.1 MTT concentration | 50 |
| 3.1.2 Time of MTT and DMSO incubation | 50 |
| 3.1.3 Number of L1 larvae | 54 |
| 3.2 Application of the MTT assay to evaluate the viability of <i>C. elegans</i> following toxicant exposure | 54 |
| 3.2.1 Exposure to ivermectin..... | 54 |
| 3.2.2 Exposure to short chain alcohols..... | 58 |
| 3.3 Application of the MTT assay to evaluate the viability of <i>H. contortus</i> following toxicant exposure | 58 |
| 3.4 Discussion..... | 58 |
| | |
| Chapter 4: A model of ivermectin resistance in nematodes | 64 |
| 4.1 Development of a model of ivermectin resistance in <i>C. elegans</i> | 65 |
| 4.2 Characterisation of resistance to ivermectin | 66 |
| 4.3 Effect of resistance on worm morphology and phenotype | 66 |
| 4.4 Cross-resistance to other anthelmintics | 70 |
| 4.5 Reversing resistance to ivermectin | 70 |
| 4.5.1 Inhibition of ABC transport protein activity | 70 |
| 4.5.2 Knockdown of multidrug resistance-associated proteins using RNA interference | 73 |
| 4.5.2.1 Design of probes to knockdown to MRPs: mrp-1 and mrp-6 | 75 |
| 4.5.2.2 Knockdown of mrp-1 and mrp-6 | 76 |
| 4.6 Discussion..... | 78 |
| | |
| Chapter 5: Changes in gene expression in ivermectin resistant nematodes.. | 83 |
| 5.1 Analysis of ABC transport proteins in <i>C. elegans</i> | 84 |
| 5.2 Design of a qRT-PCR assay to evaluate the expression of genes associated with MDR in <i>C. elegans</i> | 86 |
| 5.2.1 Oligonucleotide design | 86 |
| 5.2.3 Validation of the qRT-PCR assay | 87 |
| 5.3 Expression of ABC transport proteins and associated genes in the life stages of <i>C. elegans</i> | 88 |
| 5.4 Expression of ABC transport proteins in ivermectin resistant worms | 88 |
| (i) Cultured with ivermectin..... | 88 |
| (ii) Cultured without ivermectin (12 weeks)..... | 92 |
| (i) Returned to ivermectin (2 weeks)..... | 92 |
| 5.5 Glutathione metabolism | 95 |
| 5.5.1 Total intracellular glutathione levels..... | 95 |
| 5.5.2 Expression of <i>gcs-1</i> and <i>gstp-1</i> | 95 |
| 5.6 Response to ivermectin | 98 |
| 5.7 Discussion..... | 101 |

| | |
|---|------------|
| Chapter 6: Interaction of ivermectin & other anthelmintics with human P-glycoprotein & MRP-1 | 107 |
| 6.1 Uptake of ivermectin by cells..... | 109 |
| 6.2 Resistance to ivermectin | 109 |
| 6.3 Competition with resistance to daunorubicin and taxol | 112 |
| 6.4 Effect of ivermectin on the accumulation of P-glycoprotein and MRP-1 fluorescent substrates | 115 |
| 6.5 The interaction of other anthelmintics with P-glycoprotein and MRP-1..... | 119 |
| 6.6 Discussion | 122 |
| | |
| Chapter 7: The multidrug resistance-associated proteins of <i>H. contortus</i>... | 130 |
| 7.1 Identification of the multidrug resistance-associated proteins of <i>H. contortus</i> | 131 |
| 7.2 Design of a qRT-PCR assay to analyse the expression of the MRPs of <i>H. contortus</i> | 137 |
| 7.3 Analysis of the expression of MRPs from <i>H. contortus</i> | 142 |
| 7.4 Discussion..... | 146 |
| | |
| Chapter 8: Discussion, conclusions & future directions | 150 |
| | |
| References | 161 |

ABSTRACT

Widespread resistance to chemotherapeutic agents is one of the biggest challenges facing human health and the agricultural industry, with resistance to all current anthelmintics now recorded. Understanding the development of drug resistance in parasitic nematodes is critical to prolonging the efficacy of current anthelmintics, developing markers for monitoring drug resistance and is beneficial in the design of new chemotherapeutic agents or targets. Multidrug resistance (MDR) is mediated by ATP-binding cassette (ABC) transport proteins including the multidrug resistance-associated proteins (MRPs) and P-glycoproteins, which confer resistance to structurally and functionally different drugs. This work characterizes the role of these proteins in drug resistance in nematodes.

Using the model nematode *Caenorhabditis elegans*, ivermectin resistant sublines were developed through step-wise exposure to increasing concentrations of ivermectin commencing with a non-toxic concentration of 1 ng/ml. Resistant strains displayed a MDR phenotype with cross-resistance not only to the related drug moxidectin, but also to other unrelated anthelmintics, levamisole, pyrantel and thiabendazole but not to albendazole. Resistance was stable after 3 months without ivermectin treatment.

Resistance to low levels of ivermectin (≤ 6 ng/ml) was associated with increased expression of *mrp-1*, *mrp-6* and *pgp-1* and decreased glutathione, while higher level resistance (10 ng/ml) was primarily associated with the increased expression of P-glycoproteins. This resistance to ivermectin was reversible by the co-administration of MRP, P-glycoprotein and glutathione synthesis inhibitors confirming the involvement of these proteins in resistance. To show the relevance of this model, homologues of MRPs were identified in the gastrointestinal parasitic nematode of ruminants *Haemonchus contortus*. Increased expression of several MRPs identified in *H. contortus* was found in ivermectin resistant isolates, supporting the relevance of the *C. elegans* model.

The interaction of ivermectin with human P-glycoprotein and MRP-1 was also examined. Ivermectin clearly inhibits the transport of P-glycoprotein substrates and can reverse resistance to both daunorubicin and taxol in resistant cells. An interaction with mammalian MRP-1 is less clear, with a 10-fold lower affinity. Therefore ivermectin and modulators of P-glycoprotein have the potential to interfere with the biodisposition and bioavailability of

anthelmintics within parasitic hosts as well as parasites. Overall, this work demonstrated that low doses of ivermectin can induce resistance in nematodes through the increased expression of multidrug resistance transport proteins, adding further complexity of the development of drug resistance, and demonstrating the multi-factorial nature of MDR.

JOURNAL PUBLICATIONS

James CE & Davey, MW (2009) Increased expression of ABC transport proteins is associated with ivermectin resistance in the model nematode *Caenorhabditis elegans*. *Int J Parasitol*, **39**: 213-220.

James CE & Davey, MW (2007) A rapid colorimetric assay for the quantitation of the viability of free-living larvae of nematodes in vitro. *Parasitol Res*, **101**(4): 975-80.

PRESENTATIONS

James CE, Davey MW (2008) Multidrug resistance: It's as easy as ABC. RNSH/UTS/USYD/KIMR Scientific Research Meeting, Gore Hill NSW, Australia

James CE, Prichard RK, Davey MW (2008) Multidrug resistance-associated proteins in drug-resistant nematodes. ARC/NHMRC Network for Parasitology/ASP Conference, Glenelg SA, Australia

James CE, Davey MW (2008) ABC transporter-mediated ivermectin resistance in the model nematode *Caenorhabditis elegans* confers cross-resistance to other anthelmintics. Australasian *C. elegans* Meeting, Kioloa NSW, Australia

James CE, Davey MW (2007) Drug-efflux mediated resistance to anthelmintics in nematodes. ARC/NHMRC Network for Parasitology/ASP Conference, Canberra ACT, Australia

James CE, Davey MW (2006) Transport proteins in drug resistant nematodes. AWI Postgraduate Conference, Perth WA, Australia

James CE, Witcombe D, Davey MW (2006) Transport proteins in drug resistant nematodes. International Congress of Parasitology, Glasgow Scotland, United Kingdom.

James CE, Witcombe D, Davey MW (2005) Transport proteins as drug targets in parasitic nematodes. AWI/Sheep CRC Postgraduate Conference, Noosa QLD, Australia

LIST OF ABBREVIATIONS

| Abbreviation | Full name |
|------------------|--|
| aa | amino acid |
| AAD | amino-acetonitrile derivative |
| ABC | adenosine tri-phosphate binding cassette |
| ACh | acetylcholine |
| ATP | adenosine tri-phosphate |
| BLAST | basic local alignment search tool |
| bp | base pairs |
| BSA | bovine serum albumin |
| BSO | buthionine sulfoxamine |
| cDNA | complementary deoxyribonucleic acid |
| C _T | cycle threshold |
| DMSO | dimethyl-sulfoxide |
| DNA | deoxyribonucleic acid |
| dNTPs | deoxyribonucleotide triphosphates |
| dsRNA | double stranded RNA |
| DTNB | 5,5'-dithio-bis (2-nitrobenzoic acid) |
| E | efficiency |
| ETDA | ethylene diamine tetra acetic acid |
| g | centrifugal force (gravity) |
| GABA | gamma-amino butyric acid |
| GluCl | glutamate-gated chloride channel |
| GSH | reduced glutathione |
| GSSG | oxidized glutathione |
| GST | glutathione S-transferase |
| GS-X | glutathione-conjugate |
| h | hour |
| IC ₅₀ | inhibitory concentration (50 %) |
| IPTG | isopropylthiogalactopyranoside |
| IVR | ivermectin resistant |
| kb | kilobases |

| | |
|------------------|--|
| kDa | kilodaltons |
| L1 | first larval stage |
| L2 | second larval stage |
| L3 | third larval stage |
| L4 | fourth larval stage |
| LB | Luria Bertani Broth |
| LDA | larval development assay |
| LTC ₄ | leukotriene C ₄ |
| m | metre |
| M | molar |
| MDR | multidrug resistance |
| min | minute |
| ml | milliliter |
| MOPS | 3-(N-morpholino)propane-sulphonic acid |
| mRNA | messenger ribonucleic acid |
| MRP | multidrug resistance associated protein |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide |
| mw | molecular weight |
| nAChR | nicotinic acetyl choline receptor |
| NADPH | nicotinamide adenine dinucleotide phosphate |
| NBD | nucleotide binding domain |
| ng | nanogram |
| NGM | nematode growth medium |
| OC | degrees Celsius |
| PBS | phosphate buffered saline |
| PCR | polymerase chain reaction |
| Pgp | P-glycoprotein |
| q | quantitative |
| RNA | ribonucleic acid |
| RNAi | RNA interference |
| ROX | 6-carboxyl-X-rhodamine |
| rpm | revolutions per minute |

| | |
|----------------|---|
| RT-PCR | reverse transcriptase polymerase chain reaction |
| siRNA | short interfering ribonucleic acid |
| SNP | single nucleotide polymorphism |
| SSA | sulphosalicylic acid |
| TAE | tris-acetate-ethylene diamine tetra acetic acid |
| TFB | transformation buffer |
| T _m | melting temperature |
| TMD | transmembrane domain |
| TSH | trypanothione |
| UV | ultraviolet |
| γGCS | γ-glutamyl cysteine synthetase |

LIST OF FIGURES

Chapter 1 Introduction

| | | |
|------------|--|----|
| Figure 1.1 | Lifecycle of <i>Haemonchus contortus</i> | 3 |
| Figure 1.2 | The development of resistance to anthelmintics..... | 7 |
| Figure 1.3 | Schematic of the structure of ABC transport proteins | 13 |
| Figure 1.4 | Thiol and MRP transport systems | 16 |
| Figure 1.5 | The life cycle of <i>C. elegans</i> at 25 °C | 25 |
| Figure 1.6 | Phylogeny of nematodes | 26 |

Chapter 2 Materials and methods

| | | |
|------------|--|----|
| Figure 2.1 | BLASTN taxonomy report for pcb19 negative control RNAi probe | 43 |
|------------|--|----|

Chapter 3 A rapid colorimetric assay for the quantitation of the viability of the free-living larvae of nematodes *in vitro*

| | | |
|------------|---|----|
| Figure 3.1 | Formazan production with increasing concentrations of MTT | 51 |
| Figure 3.2 | Formazan production over time | 52 |
| Figure 3.3 | Microscopic observation of formazan production in L1 larvae of <i>C. elegans</i> | 53 |
| Figure 3.4 | Effect of time of DMSO incubation on the production of formazan | 55 |
| Figure 3.5 | Linearity of formazan production in L1 larvae of <i>C. elegans</i> and <i>H. contortus</i> | 56 |
| Figure 3.6 | Effect of ivermectin exposure on viability of <i>C. elegans</i> | 57 |
| Figure 3.7 | Viability of <i>C. elegans</i> following exposure to methanol, ethanol, 1-butanol or iso-propanol | 59 |
| Figure 3.8 | Toxicity of pyrantel and ivermectin to <i>H. contortus</i> L1 larvae | 60 |

Chapter 4 A model of ivermectin resistance in nematodes

| | | |
|------------|--|----|
| Figure 4.1 | Development of ivermectin resistant <i>C. elegans</i> following step-wise exposure to ivermectin | 67 |
| Figure 4.2 | Viability of <i>C. elegans</i> following ivermectin treatment | 68 |
| Figure 4.3 | Growth of Bristol N2 and ivermectin resistant <i>C. elegans</i> | 69 |
| Figure 4.4 | Mean worm velocity of Bristol N2 and ivermectin resistant <i>C. elegans</i> | 71 |
| Figure 4.5 | Fold resistance to anthelmintics in IVR worms | 72 |
| Figure 4.6 | Reversal of ivermectin resistance | 74 |
| Figure 4.7 | RNAi of MRPs in Bristol N2, IVR6 and IVR10 | 77 |
| Figure 4.8 | Effect of RNAi of MRPs in IVR sublines on ivermectin resistance | 79 |

Chapter 5 Expression of genes associated with MDR in ivermectin resistant *C. elegans*

| | | |
|------------|--|----|
| Figure 5.1 | Validation of primers for the $\Delta\Delta C_T$ method of relative qRT-PCR quantification | 89 |
| Figure 5.2 | MDR-associated gene expression in the life stages of Bristol N2 <i>C. elegans</i> | 90 |
| Figure 5.3 | ABC transport protein gene expression in ivermectin resistant <i>C. elegans</i> | 91 |

| | | |
|------------|---|-----|
| Figure 5.4 | ABC transport protein gene expression in ivermectin resistant <i>C. elegans</i> cultured without ivermectin | 93 |
| Figure 5.5 | ABC transport protein gene expression in ivermectin resistant <i>C. elegans</i> returned to ivermectin | 94 |
| Figure 5.6 | Intracellular glutathione levels in ivermectin resistant <i>C. elegans</i> | 96 |
| Figure 5.7 | Expression of <i>gcs-1</i> and <i>gstp-1</i> mRNA in ivermectin resistant <i>C. elegans</i> | 97 |
| Figure 5.8 | Bristol N2 mRNA fold changes in response to 24 h treatment with 6 ng/ml ivermectin | 99 |
| Figure 5.9 | IVR6 mRNA fold changes in response to 24 h treatment with 10 ng/ml ivermectin | 100 |

Chapter 6 Interaction of ivermectin & other anthelmintics with human P-glycoprotein and MRP-1

| | | |
|------------|---|-----|
| Figure 6.1 | Time course uptake of BODIPY-ivermectin by cells | 110 |
| Figure 6.2 | Toxicity of ivermectin in the CEM, CEM-VLB ₁₀₀ and CEM-E1000 cells | 111 |
| Figure 6.3 | Effect of verapamil on daunorubicin and taxol resistance | 113 |
| Figure 6.4 | Effect of ivermectin on daunorubicin and taxol resistance | 114 |
| Figure 6.5 | Effect of ivermectin on Rhodamine 123 and daunorubicin accumulation | 116 |
| Figure 6.6 | Effect of ivermectin on calcein and BODIPY-ivermectin accumulation | 118 |
| Figure 6.7 | Effect of anthelmintics on Rhodamine 123 accumulation | 120 |
| Figure 6.8 | Effect of anthelmintics on daunorubicin resistance | 121 |

Chapter 7 The multidrug resistance associated proteins of *Haemonchus contortus*

| | | |
|------------|---|-----|
| Figure 7.1 | ClustalW alignment of the partial protein sequence of nucleotide binding domain 2 of <i>C. elegans</i> and <i>H. sapiens</i> MRPs and P-glycoproteins | 133 |
| Figure 7.2 | Agarose gel electrophoresis of 3' RACE PCR with NBD2 primers | 134 |
| Figure 7.3 | Alignments of RACE derived sequences | 136 |
| Figure 7.4 | Agarose gel electrophoresis of <i>C. elegans</i> gene-specific primer products from <i>H. contortus</i> cDNA | 138 |
| Figure 7.5 | Translation of <i>H. contortus</i> py09e04.yl and qRT-PCR primer locations | 139 |
| Figure 7.6 | Linear regression of C _T versus template concentration of <i>H. contortus</i> MRP qRT-PCR reactions | 141 |
| Figure 7.7 | Relative expression of MRPs in the life stages of <i>H. contortus</i> | 143 |
| Figure 7.8 | Relative expression of MRPs in <i>H. contortus</i> selected with macrocyclic lactones ... | 144 |
| Figure 7.9 | Relative expression of MRPs in field isolates of <i>H. contortus</i> | 145 |

Chapter 8 Discussion, conclusions & future directions

| | | |
|------------|---|-----|
| Figure 8.1 | Summary of the cellular changes associated with ivermectin resistance | 155 |
|------------|---|-----|

LIST OF TABLES

Chapter 1 Introduction

| | | |
|-----------|--|----|
| Table 1.1 | Anthelmintics for the treatment of parasitic worms | 4 |
| Table 1.2 | SNPs identified in benzimidazole-resistant nematodes | 9 |
| Table 1.3 | Effect of GluCl subunits on ivermectin resistance | 9 |
| Table 1.4 | The ABC transport proteins of humans | 15 |
| Table 1.5 | P-glycoproteins and MRPs in protozoan parasites | 18 |
| Table 1.6 | P-glycoproteins and MRPs in metazoan parasites | 21 |

Chapter 4 A model of ivermectin resistance in nematodes

| | | |
|-----------|--|----|
| Table 4.1 | Effect of inhibitors of drug transport on Bristol N2 viability | 73 |
| Table 4.2 | Specificity of RNAi probes determined using E-RNAi | 75 |
| Table 4.3 | Primers for the amplification of MRP RNAi probes from cDNA | 76 |

Chapter 5 Expression of genes associated with MDR in ivermectin resistant *C. elegans*

| | | |
|-----------|---|----|
| Table 5.1 | Homology of <i>C. elegans</i> full ABC transport proteins | 85 |
| Table 5.2 | Primers for quantitative real-time PCR | 87 |

Chapter 6 Interaction of ivermectin & other anthelmintics with human P-glycoprotein and MRP-1

| | | |
|-----------|--|-----|
| Table 6.1 | Relative resistance to anthelmintics | 122 |
|-----------|--|-----|

Chapter 7 The multidrug resistance associated proteins of *Haemonchus contortus*

| | | |
|-----------|---|-----|
| Table 7.1 | Identification of MRPs from Nembase2 ESTs | 132 |
| Table 7.2 | Unique sequences identified by 3' RACE PCR | 135 |
| Table 7.3 | <i>C. elegans</i> primers for the amplification of <i>H. contortus</i> MRPs | 135 |
| Table 7.4 | Primers for quantitative real-time PCR of <i>H. contortus</i> MRPs | 137 |
| Table 7.5 | <i>H. contortus</i> MRP qRT-PCR efficiencies | 140 |