



MURDOCH
UNIVERSITY
PERTH, WESTERN AUSTRALIA

Characterisation of Community-Derived *Hymenolepis* Infections in Australia

Marion G. Macnish

BSc. (Medical Science) Hons

Division of Veterinary and Biomedical Sciences

Murdoch University

Western Australia

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2001

I declare that this thesis is my own account of my research and contains as its main work which has not been submitted for a degree at any other educational institution.

.....
(Marion G. Macnish)

Abstract

Hymenolepis nana is a ubiquitous parasite, found throughout many developing and developed countries. Globally, the prevalence of *H. nana* is alarmingly high, with estimates of up to 75 million people infected. In Australia, the rates of infection have increased substantially in the last decade, from less than 20% in the early 1990's to 55 - 60% in these same communities today. Our knowledge of the epidemiology of infection of *H. nana* is hampered by the confusion surrounding the host specificity and taxonomy of this parasite. The suggestion of the existence of two separate species, *Hymenolepis nana* von Siebold 1852 and *Hymenolepis fraterna* Stiles 1906, was first proposed at the beginning of the 20th century. Despite ongoing discussions in the subsequent years it remained unclear, some 90 years later, whether there were two distinct species, that are highly host specific, or whether they were simply the same species present in both rodent and human hosts. The ongoing controversy surrounding the taxonomy of *H. nana* has not yet been resolved and remains a point of difference between the taxonomic and medical literature.

The epidemiology of infection with *H. nana* in Australian communities is not well understood as the species present in these communities has never been identified with certainty. It is not clear which form of transmission commonly occurs in Australia, whether the *H. nana* 'strain/species' present in the north-west of Western Australia is present in human and rodent hosts, or whether humans harbour their own 'strain/sub-species' of *Hymenolepis*. Furthermore, it is not known whether mice are a potential zoonotic source for transmission of *Hymenolepis* to human hosts. In this study, 51 human isolates of *H. nana* were inoculated into highly susceptible laboratory rodent species. However, these failed to develop into adult worms in all instances, including when rodent species were chemically and genetically immunosuppressed. In addition, 24 of these human isolates were also cross-tested in the flour beetle intermediate host, *Tribolium confusum*. Of these, only one isolate developed to the cysticercoid stage in beetles, yet when inoculated into laboratory rodents, the cysticercoids also failed to develop into adult stage.

Since isolates of *H. nana* infecting humans and rodents are morphologically indistinguishable, the only way they can be reliably identified is by comparing the parasite in each host using molecular criteria. In the current study, three regions of ribosomal DNA, the small subunit (18S), the first internal transcribed spacer (ITS1) and the intergenic spacer (IGS) were chosen for genetic characterisation of *Hymenolepis* spp. from rodent and human hosts from a broad geographic range. In addition, a mitochondrial gene, the cytochrome *c* oxidase subunit 1 (CO1) gene and a non-ribosomal nuclear gene, paramyosin, were characterised in a number of *Hymenolepis* isolates from different hosts.

A small PCR fragment of 369 bp, plus a larger fragment of 1223 bp, were sequenced from the 18S gene of reference isolates of *H. nana* and the rat tapeworm *H. diminuta*. Minimal sequence variation was found in the two regions of the 18S between these two morphologically distinct, phylogenetically recognised species, *H. nana* and *H. diminuta*, and this indicated that the 18S gene was too conserved for further genetic characterisation of isolates of *H. nana* from different hosts.

A large number of human isolates of *H. nana* (104) were characterised at the ITS1 using PCR-restriction length fragment polymorphism (PCR-RFLP). The profiles obtained were highly variable and often exceeded the original size of the uncut fragment. This was highly suggestive of the existence of ribosomal spacers that, whilst identical in length, were highly variable in sequence. To overcome the problems of the variable PCR-RFLP profiles, further characterisation of the ITS1, by cloning and sequencing 23 isolates of *H. nana*, was conducted and this confirmed the existence of spacers which, although similar in length (approximately 646 bp), differed in their primary sequences. The sequence differences led to the separation of the isolates into two clusters when analysed phylogenetically. This sequence variation was not, however, related to the host of origin of the isolate, thus was not a marker of genetic distinction between *H. nana* from rodents and humans. Indeed, the levels of variability were often higher within an individual isolate than between isolates, regardless of whether they were collected from human or mice hosts, which was problematic for phylogenetic analysis. In addition, mixed parasite infections of *H. nana* and the rodent tapeworm *H. microstoma* were identified in four humans in this study, which was unexpected and surprising, as there have been no previous reports in the literature documenting humans as definitive hosts for this parasite. Further studies are required, however, to determine if the detection of *H. microstoma* in humans reflects a genuine, patent infection or an atypical, accidental occurrence.

Sequencing of the mitochondrial cytochrome *c* oxidase 1 gene (C01) in a number of isolates of *Hymenolepis nana* from rodents and humans identified a phylogenetically supported genetic divergence of approximately 5% between some mouse isolates compared to isolates of *H. nana* from humans. This provided evidence that the mitochondrial C01 gene was useful for identifying genetic divergences in *H. nana* that were not resolvable using nuclear loci. Despite a morphological identity between isolates of *H. nana* from rodent and human hosts, the genetic divergence observed between isolates at the mitochondrial locus was highly suggestive that *H. nana* is a species complex, or “cryptic” species (= morphologically identical yet genetically distinct). In addition, whilst not supported by high bootstrap values, a clustering of the Australian human isolates into one uniform genetic group that was phylogenetically separated from all the mouse isolates was well supported by biological data obtained in this study. To

confirm the phylogeny of the C01 tree a small segment of the nuclear gene, paramyosin, was sequenced in a number of isolates from humans and rodents. However, this gene did not provide the level of heterogeneity required to distinguish between isolates from rodent and human hosts. The high sequence conservation of the paramyosin gene characterised in this study did not refute the finding that *H. nana* may be a cryptic species that is becoming host adapted. It simply did not provide additional data to that already obtained.

A DNA fingerprinting tool, PCR-RFLP, of the ribosomal intergenic spacer (IGS), was developed in this study in order to evaluate its usefulness in tracing particular genotypes within a community, thus determining transmission patterns of *H. nana* between rodent and human hosts. Analysis of the IGS of numerous *H. nana* isolates by PCR-RFLP identified the presence of copies of the IGS that, whilst similar in length, differed in their sequence. Similar to that observed in the ITS1, the existence of different IGS copies was found in both rodent and human isolates of *H. nana*, thus the variability was not evidence of the existence of a rodent- or human-specific genotype. Evaluation of the intergenic spacer (IGS) as a fingerprinting tool suggests that this region of DNA is too variable within individuals and thus, cannot be effectively used for the study of transmission patterns of the tapeworm *H. nana* between different hosts.

In summary, it appears that the life cycle of *H. nana* that exists in remote communities in the north-west of Western Australia is likely to involve mainly ‘human to human’ transmission. This is supported by both the biological and genetic data obtained for the mitochondrial locus in this study. The role of the intermediate hosts, such as *Tribolium* spp., in the *Hymenolepis* life cycle is still unclear, however it would appear that it may be greatly reduced in the transmission of this parasite in remote Australian communities. In the future, it is recommended that further genetic characterisation of faster evolving mitochondrial genes, and/or suitable nuclear genes be characterised in a larger number of isolates of *H. nana*. The use of techniques which can combine the characterisation of genotype and phenotype, such as proteomics, may also be highly valuable for studies on *H. nana* from different hosts.

-oOo-

TABLE OF CONTENTS

Abstract	iii
Acknowledgements	x
Acknowledgements	x
Publications	xi
1. GENERAL INTRODUCTION	1
1.1. Classification and Nomenclature	1
1.2. Morphology and Physiology of the Adult Worm	4
1.3. Life Cycle	7
1.3.1. Direct	7
1.3.2. Indirect	9
1.4. Transmission	10
1.5. Cross-Transmission Studies Between Human and Rodent Isolates	13
1.6. Diagnosis	14
1.7. Prevalence	14
1.8. Symptoms	16
1.9. Clinical Features and Pathogenesis	17
1.10. Effects of Immunosuppression	18
1.11. Prevention and Control	20
1.12. Molecular Approaches to Genetic Characterisation of <i>Hymenolepis nana</i>	24
1.12.1. Limitations Associated with Molecular Approaches	25
1.12.2. PCR-Based Approaches	26
1.13. Choosing the Most Appropriate Regions of DNA for Molecular Characterisation	28
1.13.1. Ribosomal Genes	28
1.13.2. Mitochondrial Genes	31
1.14. Hypotheses and Aims	33
1.14.1. Aims	33
1.15. Definition of Terms	34
2. GENERAL MATERIALS AND METHODS	37
2.1. Collection of Parasite Material	37
2.1.1. Rodent	37
2.1.2. Human	38
2.2. Detection of <i>Hymenolepis</i> Eggs in Faeces Using Zinc-Sulphate Flotation	38
2.3. PCR Amplification of DNA and Automated Sequencing	38
2.4. Agarose Gel Electrophoresis	40
2.5. Cloning of PCR Products and Recombinant Clone Screening	40
2.6. Purification of Plasmid DNA	42
3. IN VIVO INFECTION TRIALS OF HUMAN ISOLATES OF HYMENOLEPIS NANA IN INSECT AND RODENT SPECIES	44
3.1. Introduction	44
3.2. Materials and Methods	45
3.2.1. Source of Parasites	45
3.2.2. Infection of Mice and Beetles Using Laboratory 'Reference' Strain	46
3.2.3. Isolation of <i>H. nana</i> Eggs from Human Faeces by Saturated NaCl Flotation for Inoculation into Rodent Hosts	47
3.2.4. Inoculation of Rodents with <i>Hymenolepis</i> Eggs and Cysticercoids	47
3.2.5. Infection Methods Tested Using Human Isolates	47
3.2.5.1. In Mice	48
3.2.5.2. In Beetles	48
3.2.6. Cortisone Acetate Treatment	49
3.2.6.1. Mice	49
3.2.6.2. Rats	50
3.2.6.3. Hamsters	50
3.2.7. Viability Tests	50
3.2.7.1. Trypsin Digestion	50
3.2.7.2. Nucleic Acid Dyes	51
3.3. Results	52
3.3.1. Infection Trials Using a Japanese Laboratory Reference Isolate in Mice and Beetles	52
3.3.2. Infection Trials of Human Isolates in Mice and Rats	52
3.3.3. Infection Trial of Mice, Rats and Hamsters Treated with Cortisone Acetate	53
3.3.4. Viability Tests Using Trypsin Digestion and Nucleic Acid Dye Staining	53
3.4. Discussion	54

4. SEQUENCING THE RIBOSOMAL DNA UNIT (18S TO 28S) IN <i>HYMENOLEPIS</i> SPECIES.	61
4.1. Introduction	61
4.2. Materials and Methods.....	63
4.2.1. Source and Collection of Parasite Material	63
4.2.2. Purification of Total DNA From Adult Worms	63
4.2.3. 18S Primer Design, PCR Amplification and Sequencing of a Small 18S Gene Product (369 bp)	64
4.2.4. Primer Design, PCR Amplification and Sequencing of Entire rDNA Unit (18S – 28S).....	65
4.3. Results	66
4.3.1. Sequence Analysis of the Small 18S Product (369 bp).....	66
4.3.2. Sequence Analysis of the Entire rDNA Unit (18S – 28S).....	67
4.3.3. Intra- and Inter-Individual Variation	67
4.4. Discussion.....	69
4.5. Appendix	71
5. EVALUATION OF DNA EXTRACTION TECHNIQUES.....	75
5.1. Introduction	75
5.2. Materials and Methods.....	76
5.2.1. Source and Collection of Parasite Material	76
5.2.2. Extraction of DNA From Adult Worms (Positive Controls).....	77
5.2.3. Pre-Treatment of Human Faecal Samples Prior to DNA Extraction	77
5.2.4. Extraction of Total DNA From Human Faeces.....	78
5.2.4.1. Method 1 (PVPP+ Glass Milk).....	78
5.2.4.2. Method 2 (Lysis Buffer + Proteinase K + Glass Milk).....	78
5.2.4.3. Method 3 (N-Cetyl-N,N,N-trimethyl-ammoniumbromide (CTAB)).....	79
5.2.4.4. Method 4 (CTAB + ProCipitate™)	79
5.2.4.5. Method 5 (Chelex® + Phenol/Chloroform + NaAc)	80
5.2.4.6. Method 6 (Chelex® + ProCipitate™ + NaAc/EtOH).....	81
5.2.5. Extraction of Total DNA From Mouse Faeces.....	81
5.2.5.1. Method 7 (Lysis Buffer + Glass Milk)	81
5.2.6. Design of PCR Primers	82
5.2.7. Specificity and Inhibition Testing of PCR Primers	82
5.2.8. PCR Amplification of 249 bp Fragment	83
5.3. Results	83
5.3.1. PCR Amplification of Hymenolepis DNA From Human Faeces.....	83
5.3.2. PCR Amplification of Hymenolepis DNA From Mouse Faeces.....	83
5.3.3. Specificity and Inhibition of PCR Primers.....	84
5.4. Discussion.....	85
6. PHYLOGENETIC ANALYSIS OF THE RIBOSOMAL ITS1 AND MITOCHONDRIAL C01 GENES IN <i>HYMENOLEPIS</i>	90
6.1. Introduction	90
6.2. Materials and Methods.....	93
6.2.1. Source and Collection of Parasite Material	93
6.2.2. Purification of Genomic DNA From Adult Worms (Reference Isolates).....	93
6.2.3. Purification of Human and Mouse Faeces.....	93
6.2.4. Primer Design, PCR Amplification and Sequencing of rDNA ITS1	93
6.2.5. Specificity and Inhibition Testing of ITS1 Primers.....	95
6.2.6. PCR-RFLP of rDNA ITS1	95
6.2.7. PCR Amplification and Sequencing of Mitochondrial C01	96
6.2.8. Phylogenetic Analysis.....	97
6.3. Results	97
6.3.1. PCR-RFLP Analysis of ITS1	97
6.3.2. Sequence Analysis of ITS1	98
6.3.3. Inter and Intra-Individual Variation of the ITS1	100
6.3.4. Specificity	104
6.3.5. Phylogenetic Analysis ITS1	105
6.3.6. Sequence Analysis of C01	105
6.3.7. Inter and Intra-Individual Variation C01	108
6.3.8. Phylogenetic Analysis C01	110
6.4. Discussion.....	112
6.4.1. Phylogenetic Analysis of Ribosomal ITS1.....	114
6.4.2. Phylogenetic Analysis of Mitochondrial C01	118
7. PHYLOGENETIC CHARACTERISATION OF A THIRD GENETIC LOCI IN <i>HYMENOLEPIS</i>	122
7.1. Introduction	122

7.1.1.	Triosephosphate Isomerase	123
7.1.2.	Paramyosin	124
7.2.	Materials and Methods	125
7.2.1.	Source and Collection of Parasite Material	125
7.2.2.	Purification of DNA From Adult Worms, Human and Mouse Faeces	125
7.2.3.	TPI Primer Design	126
7.2.4.	PCR Amplification and Sequencing of TPI Products	127
7.2.4.1.	TPI-F and TPI-R1	128
7.2.4.2.	TPI-F and TPI-R2	128
7.2.5.	Primer Design for Paramyosin (Pmy) Products	128
7.2.5.1.	Nested Primers for Paramyosin	129
7.2.6.	PCR Amplification and Sequencing of Pmy Products	131
7.2.6.1.	Pmy-F and Pmy-R (840 bp)	131
7.2.6.2.	Ext-F and Ext-R (Primary Nested PCR Reaction) (700 bp)	131
7.2.6.3.	Int-F and Int R (Secondary Nested PCR Reaction) (625 bp)	131
7.2.7.	Phylogenetic and Statistical Analysis of Pmy	131
7.3.	Results	132
7.3.1.	Sequence Analysis of Triosephosphate Isomerase (TPI) PCR Products	132
7.3.2.	Sequence Analysis of Paramyosin (Pmy) PCR Products	133
7.3.3.	Inter and Intra-Individual Variation	134
7.3.4.	Phylogenetic Analysis	135
7.3.4.1.	Paramyosin	135
7.4.	Discussion	136
7.5.	Appendix	142

8. GENETIC CHARACTERISATION OF THE RIBOSOMAL INTERGENIC SPACER IN *HYMENOLEPIS* 145

8.1.	Introduction	145
8.2.	Materials and Methods	153
8.2.1.	Source and Collection of Parasite Material	153
8.2.2.	Purification of Genomic DNA From Adult Worms (Reference Isolates)	153
8.2.3.	Purification of Human and Mouse Faeces for DNA Amplification	153
8.2.4.	Primer Design for rDNA Intergenic Spacer (IGS) of <i>H. nana</i> , <i>H. diminuta</i> and <i>H. microstoma</i>	154
8.2.5.	PCR Amplification, Cloning and Sequencing of rDNA IGS of <i>H. nana</i> , <i>H. diminuta</i> and <i>H. microstoma</i>	155
8.2.6.	PCR-RFLP Primer Design and PCR Amplification of 867 bp RFLP Fragment (<i>H. nana</i> only)	157
8.2.7.	Specificity Testing of IGS PCR-RFLP Primers	158
8.2.8.	PCR-RFLP of Small IGS Fragment Generated by Nested Primers	159
8.2.9.	PCR Amplification and Sequencing of Portuguese Isolates, M26 and M27 at the Mitochondrial C01 Locus	159
8.3.	Results	160
8.3.1.	Sequence Analysis of the Entire IGS (<i>H. nana</i> , <i>H. diminuta</i> and <i>H. microstoma</i>)	160
8.3.2.	PCR-RFLP Analysis of the Small (867 bp) IGS Fragment of <i>H. nana</i> Isolates	160
8.3.2.1.	PCR-RFLP of Australian Mouse and Human Isolates	161
8.3.2.1.1.	Hha I	161
8.3.2.2.	PCR-RFLP of Portuguese and Italian Mouse Isolates	165
8.3.2.2.1.	Hha I	165
8.3.2.2.2.	Hae III	166
8.3.3.	Sequencing of the 867 bp PCR-RFLP Product of Four Isolates (H13, H16, M16, M17)	167
8.3.4.	Specificity of IGS PCR-RFLP Nested Primers	170
8.4.	Discussion	171
8.4.1.	PCR Efficiency	172
8.4.2.	Analysis of PCR-RFLP	172
8.4.3.	Analysis of the Sequence of the Small IGS-PCR-RFLP Product (867 bp) of H13, H16, M16 and M17	173
8.4.4.	Analysis of PCR-RFLP of Portuguese Mouse Isolates	174
8.4.5.	Sequence Heterogeneity in the Multi-Copy IGS	176
8.5.	Appendix	178

9. DETECTION OF THE RODENT TAPEWORM *HYMENOLEPIS MICROSTOMA* IN HUMANS. EVIDENCE FOR ZOONOTIC TRANSMISSION? 185

9.1.	Introduction	185
9.2.	Materials and Methods	186
9.2.1.	Source and Collection of Parasite Material	186
9.2.2.	Purification of DNA From Adult Worms	186
9.2.3.	Purification of DNA From Human and Mouse Faeces	187
9.2.4.	Primer Design, PCR Amplification and Sequencing	187

9.2.5.	PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) of ITS1.....	187
9.2.6.	Specificity Testing of ITS1 Primers.....	188
9.2.7.	Morphological Comparison of <i>H. nana</i> and <i>H. microstoma</i> Eggs by Microscopy.....	188
9.3.	Results.....	189
9.3.1.	Sequence Analysis of ITS1.....	189
9.3.2.	Intra and Inter-Individual Variation.....	190
9.3.3.	Specificity.....	191
9.3.4.	PCR-RFLP Analysis of ITS1.....	192
9.3.5.	Morphology of <i>H. nana</i> and <i>H. microstoma</i> Eggs.....	195
9.4.	Discussion.....	196
10.	GENERAL DISCUSSION	204
10.1.	Introduction.....	204
10.2.	Evaluation of the Ribosomal Genes (18S, ITS1 and IGS) as Markers of Variability Between Rodent and Human Isolates of <i>H. nana</i>	207
10.2.1.	18S Ribosomal Gene.....	207
10.2.2.	Internal Transcribed Spacer 1 (ITS1) Ribosomal Gene.....	208
10.2.2.1.	Sequence Differences Between rDNA Spacers of Ribosomal Genes.....	210
10.2.2.1.1.	Maintenance of rDNA Copies on Different Chromosomes.....	211
10.2.2.1.2.	Transposons.....	212
10.2.2.1.3.	Hybridisation Events.....	212
10.2.2.1.4.	Interbreeding.....	213
10.2.3.	Internal Transcribed Spacer 2 (ITS2).....	214
10.2.4.	Intergenic Spacer (IGS) Ribosomal Gene.....	215
10.3.	Evaluation of a Mitochondrial Gene as a Marker of Variability between Rodent and Human Isolates of <i>H. nana</i>	217
10.3.1.	Cytochrome c Oxidase Subunit 1 (C01).....	217
10.3.2.	Phylogeographical Structure of Rodent Isolates of <i>H. nana</i>	218
10.3.3.	The Influence of Ecological and Environmental Factors.....	222
10.3.4.	Host-Parasite Relationship.....	222
10.3.5.	What Constitutes a Species?.....	225
10.4.	Evaluation of the Nuclear Gene Paramyosin as a Marker of Genetic Variability.....	228
10.5.	Difficulties of PCR From Eggs Extracted From Faecal Samples.....	229
10.6.	Alternative Genes as Genetic Markers of Variability Between Isolates of <i>H. nana</i>	231
10.6.1.	Alternative Mitochondrial Genes.....	232
10.6.2.	Alternative Nuclear Genes.....	234
10.7.	Future Directions and Conclusions.....	238
10.7.1.	Whole Genome Approaches.....	238
10.7.1.1.	Representational Difference Analysis.....	240
10.7.1.2.	Microarrays.....	241
10.7.1.3.	Proteomics.....	243
11.	REFERENCES.....	246

Acknowledgements

The moment has arrived.....I am finally sitting down with a weak cappuccino at my favourite Café in Fremantle contemplating how I can adequately express the feelings that I have in finally reaching this point – the thesis hand in. I am also wondering how I can adequately express my heartfelt gratitude to a growing list of people who have helped make it all possible.

First and foremost, I would like to thank the two people who have nurtured and guided me throughout my entire PhD – my supervisors Andrew Thompson and Una Morgan. They never failed to provide me with welcome advice and positive thinking. Thankyou so much to both of you.

*It doesn't seem possible to get through this process without large amounts of technical and academic help. My most sincere appreciation is extended to Dr. Jerzy Behnke in the UK. Jerzy was an invaluable source of many things – worm specimens from field trips in Portugal, numerous taxonomy references and preserved histology specimens of *Hymenolepis*, the images of which you will find in the following pages. Jerzy has also been a major springboard for many of my crazy ideas and suggestions. I am eternally grateful for his patience in reading and responding to my long-winded emails. I would like to sincerely thank Russ Hobbs for his fantastic efforts in constructing gel photos and microscope images that are also presented in this thesis. He has made a great contribution with his brilliant computer drawing skills. Thanks also go to Russ for the long discussions we had on sticky taxonomy issues!*

I would like to thank Paul Monis in South Australia who generously helped with the phylogenetic analysis of my sequencing results. Thanks also to Clare Constantine and Alan Lymbery who helped me to understand what it all means.

I tried hard not to leave a path of destruction behind me as I emerged into the last crucial weeks before finishing but I am quite certain that those who helped me format the text and diagrams hope I never do this to them again! Pat Marshall was asked to do the impossible – and managed. She turned 50 separate files into one large document and managed to keep them there, despite the computer, not because of it. I can't thank her enough for her patience and even-temper throughout. My two buddies Louise Pallant and Carolyn Read were an ongoing source of support, food, jokes and hugs to prop me up on the final formatting and print run. Thanks heaps guys!!

*I would like to extend a warm appreciation to the best group anyone could wish to work with – the Parasitology/Pharmacology group at Murdoch University. A special thanks to Aileen Elliot for passing on any *Hymenolepis* specimens that came through the department. It's been great to be part of the team.*

As I sit here sipping my cappuccino I realise I can now get into the shower at night and not have to hop back out again to jot down that “perfect sentence” that has eluded me the entire evening. What a joy to realise I no longer need to take pen and paper into the bathroom. Ditto for when I sink under the doonah only to realise I've just composed an entire paragraph that works. I no longer have to get out of bed to write it all down!

When things got really tough and Andy started suggesting I should go home, pour myself a glass of red wine and try re-writing a couple of chapters. Meanwhile, I'm secretly realising that Andy has no idea that this whole thesis has been written with a glass of red wine at hand. OK, so as Louise suggested, I should go home, drink a strong cup of coffee, sober up and re-write that damn chapter. I hope you can tell which ones have been written on strong coffee!

Lastly, I would like to convey to the entire world what an amazing family I have. I swear I could not have got through this without them. Never again will we spend entire weekends agonising over where commas and semi colons should go in sentences. To my mother Fay, my father Ian, my sisters Barbara, Jenny and Anne and my two brothers Kim and Graham

This thesis is dedicated to them all.

Publications

Part of the work presented in this thesis has been accepted for publication or presented at scientific conferences in the following form:

Abstracts of Papers Presented at Conferences

Macnish, M. G., Morgan, U. M. and Thompson, R. C. A. (1998). Molecular characterisation of *Hymenolepis nana* in Aboriginal communities in north west Western Australia. In *73rd Annual Meeting of the American Society of Parasitologists*, p44. August 16-20, Kona, Hawaii.

Macnish, M. G., Morgan, U. M., Monis, P. T., Behnke, J. M. and Thompson, R. C. A. (1999). Genetic characterisation of three DNA loci of *Hymenolepis nana*. In *Annual Scientific Meeting of the Australian Society for Parasitology Inc.*, p26. September 26-30, Yeppoon, Queensland, Australia.

Macnish, M. G., Morgan, U. M., Behnke, J. M. and Thompson, R. C. A. (2000). Evidence for the zoonotic transmission of the rodent tapeworm *Hymenolepis microstoma* to humans. In *Joint meeting of the New Zealand Society for Parasitology and Australian Society for Parasitology*, p67. September 25-29, Wellington, New Zealand.

Journal Articles

Macnish, M. G., Morgan, U. M., Behnke, J. M. and Thompson, R. C. A. (2001). Failure to infect laboratory rodents with humans isolates of *Hymenolepis nana*. *Journal of Helminthology In Press*.

Macnish, M. G., Morgan, U. M., Monis, P. T., Behnke, J. M. and Thompson, R. C. A. (2001). A molecular phylogeny of nuclear and mitochondrial sequences in *Hymenolepis nana* (Cestoda) supports the existence of a cryptic species. *Parasitology Submitted for publication*.

Macnish, M. G., Ryan, U. M., Behnke, J. M. and Thompson, R. C. A. (2001). Detection of the rodent tapeworm *Rodentolepis Hymenolepis microstoma* in humans. A new zoonosis? *International Journal for Parasitology Submitted for publication*.