

***Brachylaima cribbi* n. sp.**

(Digenea: Brachylaimidae):

**Taxonomy, life-cycle kinetics and
infections in animals and humans.**

ANDREW R. BUTCHER, B. APP. SC.

FACULTY OF SCIENCE

SCHOOL OF MOLECULAR AND BIOMEDICAL SCIENCE

THE UNIVERSITY OF ADELAIDE

SOUTH AUSTRALIA

THIS THESIS IS PRESENTED TO THE

THE UNIVERSITY OF ADELAIDE

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

JULY 2003

TABLE OF CONTENTS

ABSTRACT	VII
AUTHOR'S STATEMENT	VIII
DEDICATION	IX
ACKNOWLEDGEMENTS	X
PUBLICATIONS	XII
CHAPTER ONE	1
INTRODUCTION AND REVIEW OF THE LITERATURE	
1. Background.....	1
2. Superfamily, Family, Subfamilies and Genera of Brachylaimids	2
3. <i>Brachylaima</i> Dujardin, 1843.....	4
4. Life-cycle	13
4.1. Life-cycle of <i>Brachylaima</i> sp. in South Australia.	15
4.1.1. Intermediate hosts	15
4.1.2. Definitive hosts	16
5. Hypothesis.....	16
6. Aims and objectives.....	16
CHAPTER TWO	17
MATERIALS AND METHODS	
1. Laboratory life-cycle experiments	17
1.1. Eggs	17
1.1.1. Optimisation of mouse faecal egg counts	17
1.2. Faecal egg counts	18
1.3. Preparation of eggs for infection of snails.....	19
1.3.1. Egg purification from mouse faeces	19
1.1.2. Egg recovery from gravid worms	19
1.1.3. Egg recovery from RPMI worm cultures	20
1.4. Assessment of egg fertility	20
1.5. Intermediate host land snails	20
1.5.1. Collection of snails, taxonomy and identification	20
1.5.2. Snail cultures	21
1.5.3. Laboratory-reared snails	22

1.5.4.	Snail dissection	22
1.5.5.	Infection of first intermediate hosts	26
1.5.6.	Quantification of cercariae emerging per hour	26
1.5.7.	Sporocyst location, distribution and quantification	26
1.5.8.	Infection of second intermediate hosts	27
1.6.	Infection of laboratory definitive hosts	27
1.6.1.	Animals	27
1.6.2.	Infection procedures	28
1.6.3.	Worm recovery	28
1.7.	Establishment of a life-cycle with eggs from an infected human	29
2.	Light microscopy	30
3.	Scanning electron microscopy.....	30
4.	<i>B. cribbi</i> enzyme-linked immunosorbent assay	31
5.	Life-cycle studies in the wild	32
5.1.	Definitive host animals in the wild.....	32
5.2.	Seasonal infection rates in South Australia	32
6.	Investigation of human infections.....	33
7.	Statistical analysis	33

CHAPTER THREE..... 34

DESCRIPTION OF THE LIFE-CYCLE STAGES OF *BRACHYLAIMA CRIBBI* N. SP.
(DIGENEA: BRACHYLAIMIDAE) DERIVED FROM EGGS RECOVERED FROM
HUMAN FAECES IN AUSTRALIA

1.	Introduction.....	34
2.	Results	35
2.1.	Description	35
2.2.	Adult worm.....	35
2.3.	Eggs	39
2.4.	Sporocyst	40
2.5.	Cercaria.....	40
2.6.	Metacercaria	42
3.	Discussion and comparison	43
4.	Etymology	49
5.	Summary.....	49

CHAPTER FOUR 50

BRACHYLAIMA CRIBBI (DIGENEA: BRACHYLAIMIDAE): SCANNING ELECTRON MICROSCOPICAL OBSERVATIONS OF LIFE-CYCLE STAGES

1. Introduction	50
2. Results	51
2.1. Adult worm.....	51
2.2. Egg.....	56
2.3. Sporocyst.....	56
2.4. Cercaria.....	56
2.5. Metacercaria	57
3. Discussion	59
4. Summary	61

CHAPTER FIVE 63

SUSCEPTIBILITY OF LABORATORY MICE TO *B. CRIBBI* INFECTION

1. Introduction	63
2. Experimental plan	64
3. Results	64
3.1. Susceptibility of outbred and inbred mice.....	64
3.2. Worm burden.....	64
4. Discussion	67
5. Summary	69

CHAPTER SIX 70

COURSE OF *B. CRIBBI* INFECTION IN IMMUNOCOMPETENT C57BL/6J MICE

1. Introduction	70
2. Infectious dose of metacercariae for C57BL/6J mice	71
2.1. Results	71
3. Influence of age and sex on the course of infection	71
3.1. Results	72
4. Re-infection of male and female C57BL/6J mice	73
4.1. Results	73
5. Worm burden, fecundity and serum antibody levels after a challenge infection	77
5.1. Results	77
6. Discussion	77

6.1. Infectious dose of metacercariae for C57BL/6J mice	77
6.2. Influence of age and sex on the course of infection	81
6.3. Re-infection challenge.....	82
7. Summary.....	84
CHAPTER SEVEN	86
COURSE OF INFECTION IN IMMUNODEFICIENT MICE	
1. Introduction.....	86
2. Susceptibility of immunodeficient mice	87
2.1. Results	87
3. Comparison of the course of infection in immunodeficient and immunocompetent mice	87
3.1. Results	87
4. Egg excretion, worm burden and fecundity in NOD SCID mice.....	89
4.1. Results	89
5. Comparison of uptake of metacercaria in immunodeficient and immunocompetent mice	91
5.1. Results	92
6. Re-infection challenge of NOD SCID mice	92
6.1. Results	92
7. Discussion.....	95
8. Summary.....	98
CHAPTER EIGHT.....	99
NATURAL AND LABORATORY SUSCEPTIBILITY OF DEFINITIVE HOST ANIMALS	
1. Introduction.....	99
2. Natural definitive hosts.....	99
2.1. Results	100
2.1.1. Birds.....	100
2.1.2. Mammals	100
2.1.3. Reptiles	100
3. Susceptibility of laboratory animals.....	102
3.1. Results	102
4. Discussion.....	103
5. Summary.....	106

CHAPTER NINE..... 107

FIRST INTERMEDIATE HOST SNAILS: NATURAL INFECTION, LABORATORY SUSCEPTIBILITY AND KINETICS OF INFECTION

1. Introduction.....	107
2. Distribution of natural first intermediate hosts	108
2.1. Results	110
3. Influence of source of eggs on development of infections.....	110
3.1. Results	112
4. Laboratory susceptibility of potential first intermediate host snails	113
4.1. Results	113
5. Pre-patent period and cercarial shedding rates	115
5.1. Results	115
6. Infectious dose of eggs for first intermediate hosts	118
6.1. Results	118
7. Duration of infectivity of eggs in mouse faeces.....	121
7.1. Results	121
8. Discussion.....	123
8.1. Susceptibility	123
8.2. Egg feeding and laboratory susceptibility of snails.....	125
8.3. Kinetics of sporocyst-infection.....	126
9. Summary	128

CHAPTER TEN 129

SECOND INTERMEDIATE HOST SNAILS: NATURAL INFECTION, LABORATORY SUSCEPTIBILITY AND KINETICS OF INFECTION

1. Introduction.....	129
2. Distribution of natural second intermediate hosts	130
2.1. Results	130
3. Comparison of methods for production of metacercarial infections	133
3.1. Results	133
4. Stage-dependant resistance to infection and cercarial auto-infection.....	135
4.1. Results	135
5. Discussion.....	137
6. Summary	140

CHAPTER ELEVEN	142
SEASONAL VARIATIONS IN SPORO CYST AND METACERCARIAL INFECTION RATES ON THE YORKE PENINSULA, SOUTH AUSTRALIA	
1. Introduction.....	142
2. Experimental design	143
3. Results	143
3.1. Climatic conditions and macrobiotic changes.....	143
3.2. <i>B. cribbi</i> infection rates	148
3.2.1. Sporocyst rates.....	148
3.2.2. Metacercaria rates and intensity of infection.....	151
4. Discussion.....	155
5. Summary.....	158
 CHAPTER TWELVE	 159
HUMAN INFECTIONS	
1. Introduction.....	159
2. Experimental plan.....	160
3. Results	160
3.1. Patient demographics.....	161
3.2. Symptoms.....	163
3.3. Source of infection	165
3.4. Duration of infection and seasonal trends	166
3.5. Treatment.....	167
3.6. Concurrent infections or other medical conditions	168
4. Discussion.....	168
5. Summary	173
 CHAPTER THIRTEEN.....	 174
GENERAL DISCUSSION	
REFERENCES	184
APPENDIX A.....	200
APPENDIX B.....	205

Abstract

Brachylaima spp. (Digenea: Brachylaimidae) are terrestrial trematodes of mammals and birds and have land snails as their first and second intermediate hosts. This thesis describes a new species of *Brachylaima* and investigates infection in both snail intermediate hosts and definitive host animals. A laboratory life-cycle was established using brachylaimid eggs recovered from the faeces of an infected human. Five species of introduced European helicid and hygromiid snails, *Theba pisana*, *Cerņuella virgata*, *Cochlicella acuta*, *Cochlicella barbara* and *Microxeromagna armillata* were susceptible first intermediate hosts. These same snails and introduced *Helix aspersa* as well as the native snails *Succinea australis* and *Strangesta gawleri* were suitable second intermediate hosts. Field and laboratory studies revealed that in addition to humans and mice, various species of birds and reptiles were also definitive hosts. On the basis of its unique morphological and life-cycle features, a new species, *Brachylaima cribbi* was described. The scanning electron microscopical appearances of the various life-cycle stages were detailed. Studies of Swiss albino outbred mice and 8 strains of inbred mice revealed that C57BL/6J mice were most susceptible to *B. cribbi* infection. The peak infection occurred 4 weeks after inoculation with metacercariae following which worms were expelled over the next few weeks. Exposure to a second infection in C57BL/6J mice did not result in accelerated expulsion of adult worms but did significantly inhibit their fecundity. In contrast, when immunodeficient NOD SCID mice were infected with *B. cribbi* metacercariae the adult worms persisted for the life span of the host mice. 6,432 land snails were collected over a distance of 3,000 km across southern Australia. Sporocyst-infected snails were found in all districts of South Australia and Victoria with the percentages of infected *T. pisana*, *C. virgata*, *C. acuta* and *C. barbara* ranging from 1.7 to 4.7%. These 4 species together with *M. armillata*, *S. australis* and *S. gawleri* were infected with metacercariae being found in 18-63% of snails and the mean number of metacercariae per infected snail ranged from 2.1 to 6.1. Laboratory studies revealed that eggs may remain viable for almost 12 months in mouse faeces. The prepatent period for a sporocyst infection is 7-10 weeks after egg ingestion. Metacercariae 7 weeks of age are capable of developing into adult worms. Detailed studies of seasonal variations in sporocyst and metacercarial infection rates were studied at 4 ecologically diverse sites on the Yorke Peninsula of South Australia. The clinical features and epidemiological circumstances of *B. cribbi* infections of 12 humans are detailed, as is their satisfactory response to treatment with praziquantel.

Author's statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give my consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

.....

Andrew R. Butcher

Dated this day Wednesday, 16th of July 2003.

Dedication

To my children, Drew, Monique and Richelle;



Collecting helicid and hygromiid snails at Murray Bridge, South Australia.

“Press on: nothing in the world can take the place of perseverance. Talent will not; nothing is more common than unsuccessful men with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent.”

Calvin Coolidge, 30th President of the United States of America (1872 - 1933)

I also thank the treating practitioners, patients and parents of infected children for their cooperation and assistance in the collection of clinical data and specimens. The support of local farmers Mr Michael Richards and Mr Richard Way for allowing me to collect snails and animals on their properties and Mr Nick Allen and Mr Sergi Kozirev for their assistance with animal collections is appreciated.

Lastly, but most importantly, I thank my family. To my wife Wendy; words can not express my appreciation for your support, companionship and love over the many years of my academic life and throughout this study. My children Drew, Monique and Richelle for helping me on the numerous field trips with the collection of snails, dissecting specimens and recording data. I also thank my parents for their encouragement and support especially in those early years when I was still finding my way in life.

Acknowledgements

I would like to express my sincere gratitude to the following colleagues, friends and family, without whom, this study would not have been possible.

Firstly my supervisor, Professor David Grove, who has been be a tower of strength and knowledge. His support over the years has been unwavering and he has always had an open door approach with positive constructive feedback and encouragement. I thank him sincerely for sharing his enormous depth of knowledge and skills and above all his friendship.

I pay special thanks to Ms Helen Palethorpe for her excellent technical assistance and friendship. Collecting faecal samples, dissecting countless numbers of snails, cleaning fouled snail terrariums or painstakingly counting worm eggs; not one complaint and every task performed with the same degree of precision and accuracy.

I would like to acknowledge the support and unending tolerance of my colleagues in the Department of Clinical Microbiology and Infectious Diseases at the Queen Elizabeth Hospital. There were many times when I was so engrossed in my experiments or writing that I was oblivious to the daily dramas of a busy diagnostic laboratory. I thank them for giving me the time to do this work. I also thank the Institute of Medical and Veterinary Science and the Queen Elizabeth Hospital Pathology Advisory Fund for financial support and for allowing me the time to undertake this research.

My sincere thanks are extended to all my scientific colleagues who willingly shared their knowledge and skills to help me with this study. I especially acknowledge Dr Thomas Cribb, University of Queensland, Dr Mercedes Gracenea and Ms Olga Gonzalez-Moreno, University of Barcelona, and Professor Dr Santiago Mas-Coma, Dr Maria Bargues and Dr Adele Valero, University of Valencia, for sharing their knowledge of trematodes and particularly brachylaimids. I also acknowledge the assistance of the staff of the South Australian Natural Science Museum, in particular Dr Bob Hamilton-Bruce for mollusc identification and Dr Sylvie Pichelin and Dr Jan Forrest for assistant with the Australian Helminth Collection. Also, scientists from the South Australian Research and Development Institute in the Departments of Parasitology (Dr Ian Carmichael and Mr Michael O'Callaghan) and Entomology (Dr Suzanne Charwat, Dr Dennis Hopkins and Ms Angela Lush) who supplied specimens of parasites and snails.

Publications

During the course of this study a number of papers have been published in the scientific literature (Appendix B).

- Butcher, A.R. & Grove, D.I. (2001). Description of the life-cycle stages of *Brachylaima cribbi* n. sp. (Digenea: Brachylaimidae) derived from eggs recovered from human faeces in Australia. *Systematic Parasitology* **49**: 211-221.
- Butcher, A.R., Brealey, J.K., Grove, D.I. & Dymock, R.B. (2002). *Brachylaima cribbi* (Digenea: Brachylaimidae): Scanning electron microscopical observations of life-cycle stages. *Journal of Helminthology* **76**: 207-215.
- Butcher, A.R., Palethorpe, H.M. & Grove, D.I. (2002). The susceptibility of inbred mice to infection with *Brachylaima cribbi* (Digenea: Brachylaimidae). *Parasitology International* **51**: 109-115.
- Butcher, A.R., Palethorpe, H.M. & Grove, D.I. (2002). Effects of sex and age on the susceptibility of C57BL/6J mice to infection with *Brachylaima cribbi* and the course of infection in NOD SCID mice. *Parasitology Research* **88**: 668-674.
- Butcher, A.R. & Grove, D.I. (2003). Field prevalence and laboratory susceptibility of southern Australian land snails to *Brachylaima cribbi* sporocyst infection. *Parasite* **10**: 119-125.
- Butcher, A.R., Palethorpe, H.M. & Grove, D.I. (2003). Response to re-infection with *Brachylaima cribbi* in immunocompetent and immunodeficient mice. *Parasitology International* **52**: 219-228.