

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

A STUDY OF COCCIDIAL PARASITES

IN THE HIHI (*NOTIOMYSTIS CINCTA*)

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF VETERINARY SCIENCE
AT MASSEY UNIVERSITY

CAROLINE MILLICENT TWENTYMAN

MARCH, 2001

ABSTRACT

A systemic protozoal disease resembling atoxoplasmosis has been found to be a serious problem in the captive hihi population at the National Wildlife Centre (N.W.C.), Mt Bruce, Masterton, causing high juvenile mortality. The literature on the Genus *Atoxoplasma* is reviewed, with attention focusing on the taxonomy, history, and life cycle of the organism, named and unnamed species, identification, epidemiology and clinical signs of infection. *Atoxoplasma*-like organisms have been recognized in birds since 1900 but difficulties in identification and in classification have meant that the genus is still inadequately defined and poorly understood.

Monitoring of oocyst shedding from captive hihi at the N.W.C. during the 1997-1998 and 1998-1999 breeding seasons confirmed that the most consistent shedding was by the chicks/juveniles which had at least two periods of shedding: one in the nestling stage and one post-fledging. The earliest recorded excretion was at 9 days of age. Post-fledging, there was a period of high oocyst shedding between 6.5-8 weeks of age during both seasons. Some chicks had intermittent periods of excretion of high numbers of oocysts throughout the year although the months of December through to, and including, February were the times when high numbers of oocysts were shed by the chicks most consistently.

The adult hihi at the N.W.C. passed oocysts only sporadically, with the exception of one hand-reared bird which had little exposure to conspecifics as a juvenile, and another bird that was in poor health at the time of shedding. Small numbers of coccidial oocysts were also present in faeces collected from hihi on Tiritiri Matangi and Mokoia Islands but, largely because of infrequent sampling, no shedding patterns were discernible. It is proposed that hihi normally develop immunity to this coccidial organism as they mature if they are reared naturally, but might shed oocysts if suffering from concurrent disease.

Treatment with toltrazuril (Baycox solution 2.5%, Bayer) eliminated the shedding of oocysts in all birds. However, oocyst numbers sometimes rose again very quickly

suggesting that toltrazuril is effective against the intestinal forms of this coccidia but not against the extra-intestinal forms.

Difficulties were experienced in the *in vitro* sporulation of oocysts shed by birds from the N.W.C. although those recovered from the two islands sporulated relatively easily. The reasons for this were not established but it is suggested that the sporulation difficulties may have been due to management factors at the captive institution, such as the use of some medications. Preliminary morphological characteristics of sporulated oocysts of the *Isospora*-type are described. Two main types of coccidia were identified: Group A which comprised coccidia which had subspherical oocysts, and Group B which had ellipsoidal oocysts. Both types of coccidia were found in birds from all three locations.

These preliminary epidemiological studies suggest that infection is maintained in chicks and juveniles with oocysts remaining viable in the environment for extended periods of time. Further work on oocyst shedding by adults during the breeding and oocysts viability in the environment is required in order to confirm this hypothesis.

Transmission studies using starlings as recipient birds for both starling and hihi oocysts were not completed because of the unavailability of appropriate infective material at the required time. Another study using a single hihi as the recipient of sporulated hihi oocysts was also not completed because of the death of the hihi due to a fungal infection. A transmission study where sporulated hihi oocysts were inoculated into zebra finches, was completed and there was no evidence of infection, supporting the belief that these coccidia are species-specific.

The gross and histological findings on necropsy of 12 cases of coccidial infection in hihi from the N.W.C. are described in detail including the locations of the various coccidial forms within the body. These findings are compared with cases of *Atoxoplasma* and *Atoxoplasma*-like infections in birds recorded in the literature. The most outstanding feature of the infection in hihi is the intestinal pathology which involves extreme

thickening of the lamina propria with an overwhelming invasion by coccidial forms into the lamina propria and the intestinal epithelial cells. No atoxoplasmosis cases in other avian species exhibit similar intestinal pathology. Although there are some common aspects in the hepatic and splenic pathology, and in the tissue location of the different coccidial life cycle stages, there is currently insufficient consistent similarity to justify placing the hihi coccidia in the Genus *Atoxoplasma*. The taxonomic classification of this coccidia therefore remains uncertain.

ACKNOWLEDGEMENTS

There are numerous people who have assisted me in various ways during the course of my research. I would particularly like to thank my Chief Supervisor, Associate Professor Maurice Alley for his encouragement, help and advice during the research and the preparation of the manuscript. Special thanks are also due to my other Supervisors: Associate Professor Tony Charleston and Dr Pdraig Duignan, for their interest and contribution.

The staff at the National Wildlife Centre, Mt Bruce, most particularly Rose Collen and Glen Holland (now Curator of the Auckland Zoo), were always happy and willing to provide as much study material and help as I required. Their meticulous record-keeping and thorough management made my task that much easier and I am very grateful for their involvement in this work.

Special thanks are also due to Professor Peter Stockdale, past Dean of the Faculty of Veterinary Science, Massey University, for encouraging my interest in wildlife pathology and being such an enthusiastic early mentor.

I also wish to thank those in the parasitology group at Massey University: Dr Bill Pomroy, Shirley Calder, and Barbara Adlington, who all taught me and assisted me with the parasitology examinations and interpretations over the entire period of research.

Others to whom my thanks are due include: Shaarina and Jason Taylor, and Richard Griffiths, all of the Department of Conservation, and Dr Isabel Castro, all of whom submitted hihi samples for the research; Dr Phil McKenna of AgriQuality, Palmerston North (formerly Ministry of Agriculture and Forestries) for parasitological advice and data; Peter Russell from the Palmerston North City Council for providing many of the finches; Professor Aggie Fernando, University of Guelph, Canada, for her hospitality and sharing of her vast knowledge on coccidia; Pam Slack and Pat Davey for the histological

processing; my father, Phil Twentyman, for building all the nestboxes; Debbie Anthony for all her help with “Laurie” and the finch transmission experiment; Dr Chai Yew-Fai for advice and help with egg candling and artificial incubation techniques; Dorothy Alley for monitoring nestboxes; and Dr Jerry Pauli for accomodating my involvement with the hihi at the National Wildlife Centre and sharing his knowledge.

I wish to acknowledge with gratitude the support of the Joan Berry and Muriel Caddie Fellowships in Veterinary Science, which contributed to the funding of this research. I also wish to acknowledge the Maritime Safety Authority and the Department of Conservation for their contributions.

Finally, I would like to thank my son, Henry, who provided me with lots of smiles and laughter throughout the writing of this manuscript.

TABLE OF CONTENTS

	Page
ABSTRACT	2
ACKNOWLEDGEMENTS	5
CHAPTER ONE - GENERAL INTRODUCTION AND LITERATURE REVIEW	
1.1 INTRODUCTION	14
1.2 THE GENUS <i>ATOXOPLASMA</i>	16
1.2.1 Taxonomy	16
1.2.2 History	17
1.2.3 Life Cycle	19
1.2.4 Species of <i>Atoxoplasma</i>	21
1.2.5 Identification	27
1.2.6 Epidemiology and Clinical Signs	28
CHAPTER TWO - PARASITOLOGY	
2.1 INTRODUCTION	31
2.2 MATERIALS AND METHODS	33
2.2.1 Collection of samples	33
(i) Captive birds	33
(ii) Free-living birds	35
2.2.2 Examination of samples	35
2.2.3 Cleaning and concentrating of oocysts	36
2.2.4 Procedures for attempted sporulation	37

2.2.5	Assessing sporulation and species identification	38
2.2.6	Storage of oocysts	39
2.3	RESULTS	39
2.3.1	Results of faecal examinations of hihi from the National Wildlife Centre	39
(i)	Oocyst shedding from chicks/juveniles in the 1997-1998 breeding season	39
(ii)	Oocyst shedding from adults in the 1997-1998 breeding season	42
(iii)	Oocyst shedding from chicks/juveniles in the 1998-1999 breeding season	42
(iv)	Oocyst shedding from adults in the 1998-1999 breeding season	44
(v)	Shedding of <i>Capillaria</i> eggs	46
(vi)	Oocyst shedding by the hand-reared bird, "Keith"	46
(vii)	Pre-laying to post-hatching oocyst shedding by parents of chicks	48
2.3.2	Results of faecal examinations of hihi from other localities	48
2.3.3	Results of sporulation	48
(i)	Sporulation methods	48
(ii)	Sporulation times	49
2.3.4	Oocyst morphology	51
2.4	Discussion	55

CHAPTER THREE - TRANSMISSION EXPERIMENTS

3.1	INTRODUCTION	62
3.2	MATERIALS AND METHODS	65
3.2.1	Starling Experiment	65
(i)	Examination of wild starling faeces	65
(ii)	Acquisition of eggs	65
(iii)	Incubation of eggs	65
(iv)	Raising of parasite-free nestlings	66

3.2.2	Hihi Experiment	66
(i)	Source of experimental bird	66
(ii)	Care of experimental bird	67
(iii)	Sampling	67
(iv)	Inoculation	67
3.2.3	Finch Experiment	68
(i)	Experimental birds	68
(ii)	Pre-inoculation sampling and treatment	68
(iii)	Preparation of inoculum	68
(iv)	Inoculation	69
(v)	Euthanasia and Necropsy	69
3.3	RESULTS	70
3.3.1	Starling Experiment	70
3.3.2	Hihi Experiment	70
(i)	Daily monitoring	70
(ii)	Necropsy Results	71
3.3.3	Finch Experiment	72
(i)	Daily monitoring	72
(ii)	Necropsy Results	72
3.4	DISCUSSION	73
3.4.1	Starling Experiment	73
3.4.2	Hihi Experiment	73
3.4.3	Finch Experiment	74

CHAPTER FOUR - PATHOLOGY

4.1	INTRODUCTION	75
-----	--------------	----

	10
4.2 MATERIALS AND METHODS	76
4.2.1 Source of material	76
4.2.2 Necropsy procedure	76
4.3 RESULTS	77
4.3.1 Case histories	77
4.3.2 Gross findings	78
4.3.3 Histopathology	79
4.4 DISCUSSION	93
CHAPTER FIVE - GENERAL DISCUSSION	98
REFERENCES	104
APPENDICES	110

LIST OF THE TABLES

Table		Page
1.1	Species of <i>Atoxoplasma</i>	22
2.1	Descriptive statistics of the two types of oocysts	52
3.1	Time intervals of euthanasia	69
3.2	Results of daily monitoring of "Laurie"	71
4.1	Epidemiological factors and clinical signs in affected hihi from the N.W.C.	77
4.2	Gross findings in 12 affected hihi from the N.W.C.	78
4.3	Histological findings in 12 affected hihi from the N.W.C.	82
4.4	Presence and location of coccidial organisms in 12 affected hihi from the N.W.C.	90

LIST OF FIGURES

Figure		Page
2.1	Oocyst shedding by chicks during January 1998	40
2.2	Oocyst shedding by juveniles during Feb-March 1998	41
2.3	Oocyst shedding by chicks during Dec 1998 and Jan 1999	45
2.4	Photomicrograph of large numbers of unsporulated oocysts	50
2.5	Photomicrograph of an early distorted oocyst	50
2.6	Photomicrograph of a sporulated, subspherical Type A oocyst	54
2.7	Photomicrograph of a sporulated, ellipsoidal Type B oocyst	54
4.1	Intestine of case no. 27721 showing distended and turgid hibi intestine	80
4.2	Thickness of the intestinal wall of case no. 27561	81
4.3	The intestine of case no. 27561 demonstrating the extreme thickening of the lamina propria caused by macrophage infiltration and fibroplasia	83
4.4	The intestine of case no. 26375A showing a schizont in longitudinal section within the lamina propria	83
4.5	Section of case no. 26375A showing several groups of distinct schizozoites in	

- parasitophorous vacuoles as well as several unidentified protozoal stages which are probably immature schizonts 84
- 4.6 Section of intestine of case no. 27561 showing 2 large schizonts containing 10 or more schizonts in the lamina propria 84
- 4.7 The intestine of case no. 26375A showing a large oocyst, a schizont in cross section and several schizonts in parasitophorous vacuoles 86
- 4.8 The intestine of case no. 27561 showing severe epithelial hyperplasia and the presence of large numbers of sexual coccidial stages within epithelial cells 86
- 4.9 The intestine of case no. 27561 showing the base of an epithelial gland and adjacent lamina propria with many macrogametes, a microgamete, and a possible zygote present in epithelial cells 87
- 4.10 Section of liver from case no. 26375A showing scattered multifocal areas of mixed inflammatory cell infiltration 87
- 4.11 Section of liver from case no. 26375A showing several distinct oocysts with complete oocyst walls and a schizont surrounded by its parasitophorous vacuole within a macrophage 88
- 4.12 High power section from case no. 26375A showing two oocysts within macrophages 88
- 4.13 Section of liver from case no. 27561 showing severe deposition of haemosiderin 89
- 4.14 Low power view of spleen from case no. 26375A showing severe proliferation of histiocytic cells 89
- 4.15 Section of spleen from case no. 26375A showing several schizonts within macrophages, both in longitudinal section and in transverse section 92
- 4.16 Section of kidney from case no. 27721 showing a schizont within a blood vessel in the renal interstitium 92