

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.

**An investigation of causes of disease among wild
and captive New Zealand falcons (*Falco
novaeseelandiae*), Australasian harriers (*Circus
approximans*) and moreporks (*Ninox
novaeseelandiae*).**

A thesis presented in partial fulfilment of the requirements for the
degree of

Master of Veterinary Science

at Massey University, Turitea, Palmerston North, New Zealand

Mirza Vaseem

2014

ABSTRACT

Infectious disease can play a role in the population dynamics of wildlife species. The introduction of exotic birds and mammals into New Zealand has led to the introduction of novel diseases into the New Zealand avifauna such as avian malaria and toxoplasmosis. However the role of disease in New Zealand's raptor population has not been widely reported. This study aims at investigating the presence and prevalence of disease among wild and captive New Zealand falcons (*Falco novaeseelandiae*), Australasian harrier (*Circus approximans*) and moreporks (*Ninox novaeseelandiae*).

A retrospective study of post-mortem databases (the Huia database and the Massey University post-mortem database) undertaken to determine the major causes of mortality in New Zealand's raptors between 1990 and 2014 revealed that trauma and infectious agents were the most frequently encountered causes of death in these birds. However, except for a single case report of serratospiculosis in a New Zealand falcon observed by Green et al in 2006, no other infectious agents have been reported among the country's raptors to date in the peer reviewed literature. During the review of post-mortem records, organisms like *Mycobacterium avium*, *Serratospiculum* sp, *Sarcocystis* spp, *Trichomonas gallinae* and several unidentified helminths were identified as contributing or definite causes of mortality in all three species of raptors. But neither *Plasmodium* spp nor *Toxoplasma gondii* infections have been demonstrated in these birds so far. Therefore, a separate study was designed to determine the presence of these pathogens in New Zealand falcon, Australasian harrier and morepork tissues, using established molecular techniques.

Molecular analysis of archived New Zealand raptor tissues confirmed the presence of both *Plasmodium* spp (10/117; 8.5%) and *T. gondii* (9/117; 7.7%) in all three species of raptors. *Plasmodium* strains identified were *P. elongatum* GRW6, *P. sp* AFTRU5, and *P. relictum* GRW4 and SGS1. Surprisingly, two Australasian harriers and one morepork tested for the presence of both *Plasmodium* spp and *T. gondii* as concomitant infections. However, it is unknown whether any of the positive tested birds suffered from clinical infections, since post-mortem records had no record of clinical signs of disease associated with either infections in these birds.

Once the presence of the aforementioned pathogens among New Zealand raptors was established, an attempt was made to investigate their presence among live raptor populations as well. Blood samples were collected from raptors being admitted to Wildbase Hospital, Massey University, Palmerston North and Wingspan- Birds of Prey Research Centre, Rotorua. Molecular analysis of these samples by PCR did not reveal the presence of *Plasmodium* spp in any of the

tested birds, but one New Zealand falcon, Australasian harrier and morepork each tested positive for the presence of *T. gondii*. Interestingly, none of the positive birds showed any signs of clinical illness that may be associated with toxoplasmosis in raptors. We also analysed faecal samples and throat swabs from these birds to determine the presence of pathogens like *Caryospora* spp, *Serratospiculum* spp, *Salmonella* spp and *T. gallinae*, since many of these organisms have been detected in New Zealand and are also found affecting raptors in other parts of the world. However, apart from eggs resembling *Capillaria* spp, none of the other pathogens listed above were identified.

My study has some limitations such as a small sample size and a geographic bias in terms of birds being submitted to Massey University, Palmerston North for post-mortem analysis. But this research may be regarded as the first report of *Plasmodium* spp and *T. gondii* infections among New Zealand's three well-known raptor species and further research is required to determine the prevalence of these pathogens among the country's total raptor population, pathogenicity of the organisms towards them and the role of these birds in the epidemiology of these diseases within New Zealand

DECLARATION

The studies presented in this thesis were completed by the author whilst a postgraduate student in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University. I officially state that this is my own work and the views presented are mine, and that the contents have not been submitted for any other degree. I certify that to the best of my knowledge any help received in preparing this thesis and all sources used have been acknowledged in the thesis.

Mirza Vaseem

Supervisor

Brett Gartrell

TABLE OF CONTENTS

TABLE OF CONTENTS	iv
ACKNOWLEDGEMENTS	vii
THESIS STRUCTURE AND FORMAT	ix
LIST OF TABLES	x
LIST OF FIGURES	xi
CHAPTER 1: LITERATURE REVIEW	1
1.1 New Zealand’s raptors- a short description	1
1.2 New Zealand falcon (<i>Falco novaeseelandiae</i>)	1
1.2.1 Distribution and Biology.....	2
1.2.2 Breeding.....	2
1.2.3 Diet and feeding habit.....	3
1.3 Australasian harrier (<i>Circus approximans</i>)	3
1.3.1 Distribution and Biology.....	3
1.3.2 Breeding.....	4
1.3.3 Diet and feeding habit.....	4
1.4 Morepork (<i>Ninox novaeseelandica</i>)	4
1.4.1 Distribution and Biology.....	5
1.4.2 Breeding.....	5
1.4.3 Diet and feeding habit.....	6
1.5 Diseases and threats	6
1.6 Diseases of Falconiformes and Strigiformes	7
1.6.1 Protozoa	7
1.6.2 Haemoprotozoa	12
1.6.3 Nematodes	15
1.6.4 Trematodes, Cestodes and Acanthocephalans	17
1.6.5 Bacteria	17
1.6.6 Viruses	20
1.6.7 Fungal diseases	23
1.7 Specific aims of the study	24
1.8 References	24
CHAPTER 2: RETROSPECTIVE STUDY OF POST MORTEM FINDINGS IN NEW ZEALAND’S RAPTORS	35
2.1 Abstract	35
2.2 Introduction	36
2.2 Methodology	37

2.2.1 Post mortem categories	38
2.2.2 Statistical analysis	38
2.3 Results	39
2.3.1 Overall frequency of mortality/disease.	41
2.3.2 Causes of mortality/disease in New Zealand falcons.....	42
2.3.3 Causes of mortality/disease in Australasian harriers	42
2.3.4 Causes of mortality/disease in moreporks.....	43
2.4 Discussion	44
2.5 References	47
CHAPTER 3: THE PREVALENCE OF <i>PLASMODIUM</i> SPP. AND <i>TOXOPLASMA</i>	
<i>GONDII</i> IN ARCHIVED TISSUE SAMPLES FROM NEW ZEALAND RAPTORS... 49	
3.1 Abstract.....	49
3.2 Introduction	49
3.3 Methodology.....	51
3.3.1 Samples	51
3.3.2 Molecular analyses	51
3.3.3 Statistical analysis	52
3.4 Results	52
3.4.1 Prevalence and lineages of <i>Plasmodium</i> spp.	52
3.4.2 Prevalence of <i>Toxoplasma gondii</i>	53
3.4.3 Co-infections with <i>Toxoplasma gondii</i> and <i>Plasmodium</i> spp	53
3.4.4 Statistical analysis	55
3.5 Discussion	55
3.6 References	57
CHAPTER 4: A SURVEY OF WILD AND CAPTIVE NEW ZEALAND RAPTORS	
FOR SELECTED PATHOGENS, INCLUDING <i>PLASMODIUM</i> SPP, <i>TOXOPLASMA</i>	
<i>GONDII</i>, <i>SALMONELLA</i> SPP, HELMINTH AND PROTOZOAL PARASITES. 60	
4.1 Abstract.....	60
4.2 Introduction	60
4.3 Methodology.....	61
4.3.1 Samples	61
4.3.2 Molecular analyses	62
4.3.3 Parasitological analysis of faeces	62
4.3.4 Microbiological culturing of <i>Salmonella</i> spp	63
4.3.5 Animal ethics	63
4.4 Results	63
4.4.1 Prevalence of <i>Plasmodium</i> spp.....	63
4.4.2 Prevalence of <i>T. gondii</i>	63
4.4.3 Parasitological analysis.....	63
4.4.4 Microbiological analysis.....	64
4.5 Discussion	64
4.7 References	67
CHAPTER 5: GENERAL DISCUSSION	70
5.1 Overview of major findings.....	70
5.1.1 Identification of major causes of mortality in New Zealand's raptors.....	70

5.1.2 Determination of the presence of <i>Plasmodium</i> spp and <i>Toxoplasma gondii</i> in archived New Zealand raptor tissues.....	71
5.1.3 Determination of the presence of <i>Plasmodium</i> spp and <i>T. gondii</i> in live New Zealand raptors.....	72
5.2 Scope and limitations of the study.....	73
5.3 Future implications of the study	74
5.3 References	76
APPENDIX 1: TISSUES FROM 117 RAPTORS WERE SELECTED FOR MOLECULAR ANALYSIS TO DETERMINE THE PRESENCE OF <i>PLASMODIUM</i> SPP AND <i>T. GONDII</i>	78
A1.1 New Zealand falcons	78
A1.2 Australasian harriers	80
A1.3 Morepork.....	82
APPENDIX 2: NESTED PCR PROTOCOL FOR THE DETECTION OF <i>PLASMODIUM</i> DNA IN FORMALIN-FIXED OR PARAFFIN-EMBEDDED TISSUES	84
APPENDIX 3: DETECTION OF <i>TOXOPLASMA GONDII</i> DNA IN FORMALIN-FIXED OR PARAFFIN-EMBEDDED TISSUES.....	86

ACKNOWLEDGEMENTS

I would like to take this opportunity to deliver my heartfelt appreciation to everyone who helped me put together this thesis. With special thanks to my supervisors, Brett Gartrell and Laryssa Howe, who guided me through the entire research with utmost patience in spite of my shortcomings. I'm grateful to the humble, yet immensely knowledgeable Dr. Brett, for having given me an opportunity to work with him. Every session with Brett has sent me back enlightened and motivated to learn more. There is a special place in my life for Dr. Laryssa, who has been a great mentor, a friend, a strict teacher and overall, an amazing person. I practically owe my research to this wonderful lady and shall never forget what she has taught me.

My research involved almost three months of laboratory work, which would have never finished on time if it weren't for Mrs. Liz Burrows, whose help and guidance I required quite often. I shall take this opportunity to both thank her for being there and also to apologize for having ruined her gels a couple of times.

I would like to convey my sincere gratitude to the entire team of Wildbase, Wildlife hospital, Department of Pathology and Department of Parasitology, IVABS, Massey University, Palmerston North, especially Dr. Roberto Aguilar, Dr. Stuart Hunter and Mrs. Barbara Adlington for sharing their expertise and enabling me to sail through the sampling aspect of my research with ease. I also extend my thanks to the Department of Conservation, Rotorua and the respected Iwi for granting me the necessary permissions required for the completion of my research.

The entire staff of Wingspan- Birds of Prey Research Center, Rotorua reserves a special mention in this thesis for igniting the spark of interest for raptors in me and for also allowing the sampling of their beloved birds. I have not come across a more dedicated and helpful staff than that of Wingspan and it has been an enriching experience working with Ms. Ineke Smets, Mr. Andrew Thomas and Mr. David Crimp, whose work ethics I shall emulate wherever I go.

A single day in New Zealand would be impossible to get by without the help of my dearest landlord, Mr. Stephen Phipps, whose advice and twisted sense of humor helped me unwind after a hard day, the ever so sweet Mrs. Adele Roberts, my dearest buddy Aly, the charming Angeline, Preethi, her beau Ian, my football mates, my IVABS room mates, Karlette and Dhananjay. I deeply thank every one of you for having made my stay memorable and hope to see you all again soon.

Last but not least, I thank my mother for moulding me to be what I am today.
Pray every child is blessed with a mother like you.

THESIS STRUCTURE AND FORMAT

The structure of the thesis consists of five parts, a literature review (Chapter 1) that details the various infectious diseases affecting birds of the families Falconidae, Accipitridae and Strigidae in different parts of the world and a summary of the natural history of New Zealand's raptors; a retrospective analysis of post mortem records to document the various causes of mortality in the aforementioned birds between 1994 and 2014 (Chapter 2); a chapter describing the investigation of *Plasmodium* spp and *T. gondii* in three species of New Zealand raptors through molecular analysis of archived post mortem tissues (Chapter 3); a similar chapter involving the identification of these organisms along with *Salmonella* spp, *Trichomonas gallinae*, *Serratospiculum* spp and *Caryospora* spp in live wild and captive New Zealand falcons, Australasian harriers and moreporks (Chapter 4); and finally a discussion of the research findings with comments on the general fitness of New Zealand raptors and future implications on the conservation of New Zealand falcons (Chapter 5).

The research chapters have been written with the intension to publish and as such there is some duplication in the introduction and methods sections and relevant references can be found at the end of each chapter.

LIST OF TABLES

Table 1: Various coccidia spp encountered in Accipitridae species	9
Table 2: <i>Caryospora</i> species encountered in various Falconidae species	10
Table 3: Frequency of pathological diagnosis among New Zealand's native raptors from 1991-2014.	42
Table 4: Frequency of diagnosis of infectious organisms isolated at post mortem examination from New Zealand's native raptors.....	44
Table 5: Summary of the prevalence of <i>Plasmodium</i> spp and <i>Toxoplasma gondii</i> in New Zealand falcons, Australasian harriers and moreporks in relation to their captivity status.	53
Table 6: Summary of molecular analysis on tissue samples from New Zealand raptors to investigate the presence of <i>Plasmodium</i> spp and <i>Toxoplasma gondii</i>	54
Table 7: Results of laboratory analysis conducted on blood and faecal samples from live New Zealand raptors.	64

LIST OF FIGURES

- Figure 1:** Geographical distribution and number of New Zealand falcon (*Falco novaeseelandiae*) mortalities subject to post-mortem examination in New Zealand between 1994 and 2014. 37
- Figure 2:** Geographical distribution and number of Australasian harrier (*Circus approximans*) mortalities subject to post-mortem examination in New Zealand between 1994 and 2014. 38
- Figure 3:** Geographical distribution and number of morepork (*Ninox novaeseelandiae*) mortalities subject to post-mortem examination in New Zealand between 1994 and 2014. 39