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Coccidiosis in the Kiwi
(*Apteryx* spp.): Aspects of the
Pathology, Epidemiology and
Parasite Biology

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

Doctor of Philosophy

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This thesis is dedicated to my wonderful Nana

Joan Cross

22 July 1925 - 9 June 2013

Abstract

Although coccidiosis was first reported in kiwi 35 years ago, there has been little published information available on this disease, and the following research presents the first in-depth account of coccidiosis in kiwi. In the present study, coccidiosis was found mainly in juvenile birds and affected all five species of kiwi (*Apteryx* spp.). Infection was common, with 13% of kiwi submitted for necropsy and 25% of droppings examined during the study period presenting evidence of coccidia. Disease may cause mortality, and coccidiosis was the primary diagnosis in 26% of kiwi with pathological evidence of infection. Clinical signs associated with disease were usually non-specific, and included a combination of depression, dehydration, inappetance, weight loss and diarrhoea with fresh blood or meleana. Enteric coccidiosis was the most common form identified at necropsy, with evidence of at least three separate intestinal species of coccidia in kiwi. The presence of lamina propria macromeronts and large colorectal polyps containing progametocytes suggested at least some enteric species in kiwi may be highly fecund. In addition, the life cycle of some species appears highly complex. Renal merogony and gametogony was a common finding, suggestive of a separate species of coccidia within the kidney. Visceral stages of merogony were also identified in the liver, and occasionally in the spleen and lung. It is currently unknown where gametogony occurs for these visceral forms, and whether these are obligatory parts of the life cycle of one or more coccidial species or rather aberrant presentations of those normally found in the intestine or kidney. Morphological studies confirmed that at least four of the coccidia affecting kiwi are of the genus *Eimeria*, and these findings were substantiated using molecular techniques targeting the internal transcribed spacer (ITS) regions of the coccidial ribosomal DNA. Molecular results showed significant sequence variability within both ITS-1 and ITS-2 regions, consistent with findings from *Eimeria* spp. from other hosts. Results from this research provide the foundation for future investigations into the biology of coccidia affecting kiwi, enabling sound veterinary advice for management of this disease.

Preface

This thesis consists of seven chapters, including a Literature Review (Chapter 1) and a General Discussion (Chapter 7). The experimental chapters (Chapters 2-6) are presented in the style of publishable papers. Two chapters (2 and 3) have been published; Chapter 4 has been submitted for publishing (with minor modifications to that presented here); and Chapters 5 and 6 will be published in the future. Published papers have been amended to cite additional information contained in the appendices, including standard operating procedures and further detailed results. In addition, the reference list from each chapter has been condensed into a single bibliography which has been reformatted for consistency and presented at the end of the thesis. Citations and the bibliography follow the format of the Journal of Parasitology. Each paper stands alone; therefore there is some repetition of material between chapters. Chapters are presented in the order of publication, with data in each chapter building on those previously written.

Authorship of Chapters

I was the primary author on all work in this thesis, including the published/submitted papers. Associate Professor Maurice Alley was my chief PhD supervisor, and co-supervisors were Professor Bill Pomroy, Dr. Isabel Castro and Dr. Laryssa Howe. All supervisors contributed to ideas for the chapters, and specific contributions are outlined below.

Chapter 2

This paper appears exactly as published in Parasitology Research:

Morgan, K.J., Alley, M.R., Pomroy, W.E, Castro, I., Howe, L., 2012. Enteric coccidiosis in the brown kiwi (*Apteryx mantelli*). Parasitology Research 111, 1689-1699

Contributions of co-authors:

Ass. Prof. Alley and Prof. Pomroy were both involved in the experimental design of this research and contributed significantly to interpretation of results. Ass. Prof. Alley

was instrumental in assisting with the interpretation and description of histological findings. Dr. Castro assisted in writing of the introduction and contributed to the discussion, and all co-authors commented on the draft manuscript.

Chapter 3

This paper appears exactly as published in Avian Pathology:

Morgan, K.J., Alley, M.R., Pomroy, W.E., Gartrell, B.D., Castro, I., Howe, L., 2013. Extraintestinal coccidiosis in the kiwi (*Apteryx* spp.). Avian Pathology 42, 137-146.

Contributions of co-authors:

Ass. Profs. Alley and Gartrell performed the original necropsy on a significant proportion of the cases recorded in the “Huia” database. Ass. Prof. Alley and Prof. Pomroy assisted with the experimental design and discussion, and Ass. Prof. Alley helped significantly with interpretation and descriptions of histological findings. Ass. Prof. Alley took the photograph used in Figure 1a, and Ass. Prof. Gartrell took the photograph used in Figure 2a. Dr. Castro assisted with the introduction relevant to kiwi biology, and all co-authors commented on paper drafts.

Chapter 4

A modification of this paper has been submitted to the New Zealand Veterinary Journal:

Morgan, K.J., Castro, I., Lopez-Villalobos, N., Pomroy, W.E., Alley, M.R., Gartrell, B.D., Hunter, S., Howe, L. An epidemiological study of coccidiosis in kiwi (*Apteryx* spp.). New Zealand Veterinary Journal, submitted 10 August 2013.

Contributions of co-authors:

Ass. Profs. Alley and Brett Gartrell, and Stuart Hunter performed many of the necropsies recorded in the “Huia” database that were utilised in this study. Dr. Castro assisted with the experimental design, including statistical analysis, as well as interpretation of results and discussion of findings with relevance to kiwi biology. Dr. Castro also contributed significantly to preparation of the submitted manuscript. Dr. Lopez-Villalobos designed the statistical analysis and taught me how to perform

logistical regression analysis on the data. Ass. Prof. Alley, Prof. Pomroy, Ass. Prof. Gartrell, and Drs. Castro and Howe all commented on the draft manuscript.

Chapter 5

This chapter contains information on morphology of a number of *Eimeria* spp. identified from kiwi, and it is anticipated to publish this in the future.

Contributions of supervisors:

Prof. Pomroy helped with experimental design with regards to sporulation of oocysts and techniques for morphological examination of sporulated oocysts, and contributed to interpretation of findings including grouping of oocysts into morphotypes. Dr. Howe assisted with techniques for collection of images. Ass. Prof. Alley assisted with experimental design, and all supervisors commented on the draft manuscript.

Chapter 6

This chapter provides molecular evidence of *Eimeria* spp. from the kiwi, and it is anticipated these results will be published in the future.

Contributions of supervisors:

Dr. Howe taught me all the molecular techniques utilised in this study, and assisted throughout with experimental design, interpretation of results, and troubleshooting problems. She contributed significantly to preparation of all aspects of the manuscript. Prof. Pomroy also assisted with experimental design, in particular with regards to preparation of individual oocysts for PCR, and contributed to discussions on the direction of the research. Ass. Prof. Alley and Dr. Castro also contributed to ideas for the experimental design, and all co-authors commented on the draft manuscript.

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Permitting and Animal Ethics Requirements

As there were no manipulations of live animals during this study, Massey University Animal Ethics approval was not required.

For the collection of droppings for the epidemiological study (Chapter 4), a permit was obtained from the Department of Conservation (National Permit Number: WA-23253-RES).

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Abbreviations

BLAST	Basic local alignment search tool
BNZONE	Bank of New Zealand Operation Nest Egg™
bp	Base pair
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DVC	Disseminated visceral coccidiosis
GSK	Great spotted kiwi
H&E	Haematoxylin and eosin
ITS	Internal transcribed spacer
LSK	Little spotted kiwi
Mbp	Million base pairs
MUSCLE	Multiple sequence comparison by log-expectation
NTS	Non-transcribed spacer
OPG	Oocysts per gram
PAS	Periodic acid Schiff
PCR	Polymerase chain reaction
rDNA	Ribosomal deoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
Sp.	Species (singular)
Spp.	Species (plural)
Syn.	Synonym
<i>Taq</i>	<i>Taq</i> polymerase