The *Eimeria* species affecting brown kiwi; host-parasite interactions and conservation implications

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Harry Stewart Taylor

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Unless someone like you cares a whole awful lot,

Nothing is going to get better. It’s not.

- ***The Lorax, Dr Seuss***

To Dez,

Kwa heri, rafiki. Safari salama.

(Good bye, my friend. Safe journey.)

Abstract

Brown kiwi (*Apteryx mantelli*) are a threatened flightless nocturnal ratite endemic to New Zealand. The conservation of this species currently utilises a recovery programme known as 'Operation Nest Egg' (ONE) to increase numbers of brown kiwi in the wild. However, ONE results in a high density of immunologically naïve kiwi being housed in semi-captive conditions with the potential to result in significant morbidity, and occasionally mortality, from coccidiosis caused by multiple species of *Eimeria*. The aim of this research was to describe any circadian variation in oocyst shedding occurring for the *Eimeria* spp. affecting brown kiwi. Dropping samples were collected from brown kiwi at an ONE site using video surveillance to determine the time of excrement. Oocyst counts were carried out on these droppings and analysed in relation to the time of excrement and the days since the most recent toltrazuril application. The results show that two of the *Eimeria* spp. affecting brown kiwi exhibit circadian variation in oocysts shedding. Oocyst counts for each of the one hour time slots starting at 3am, 4am, 5am and 6am were significantly ($p<0.05$) higher than each of the time slots starting at 8pm, 9pm, 10pm and 11pm. This indicates that peak oocyst shedding occurs between 3am and 7am, with few or no oocysts shed between 8pm and 12pm. The results also suggest high prevalence and abundance of *Eimeria* spp. oocysts in the droppings, with 91% of samples from during peak shedding being positive for *Eimeria* spp., despite recent toltrazuril administration. These findings have several important implications for the conservation of brown kiwi. The reported circadian variation may affect the accuracy of coccidia testing and provides insight into the evolution of this adaptive trait in coccidia. The apparent lack of efficacy of toltrazuril may have management implications and requires further research. The results of this research increase our understanding of the biology of the *Eimeria* spp. affecting brown kiwi. Continuing to improve our understanding of host-parasite interactions is vital to enable effective disease management in order to reduce the detrimental impact of coccidia on

ONE and ensure the ongoing success and sustainability of this important conservation programme.

Preface

This thesis consists of four chapters, including a literature review (Chapter One) and a General Discussion (Chapter Four). The experimental chapters are presented in the style of publishable papers. Chapter Two has been published in *Parasitology Research* (with minor modifications to that presented here), and Chapter Three will be published in the future. These papers have been amended to cite additional information in the appendices, including standard operating procedures and detailed results. In addition the reference lists have been combined into a single reference list and is presented at the end of the thesis. Citations and the reference list follow the APA 6th edition format. As each paper stands alone, there is some repetition between chapters.

Authorship of Chapters

I was the primary author on all work in this thesis, including the published and submitted papers. Dr Kerri Morgan was my chief supervisor and Professor Bill Pomroy and Kate McInnes were co-supervisors.

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Permitting and Animal Ethics Requirements

This research did not involve manipulation of live animals, thus Massey University Animal Ethics approval was not required.

As the collection of samples did not involve disturbance of kiwi a Department of Conservation permit was not required.

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Introduction

The brown kiwi (*Apteryx mantelli*) is a flightless nocturnal avian species endemic to New Zealand and is classified as at risk and in decline (Robertson et al., 2017). Intensive management is carried out by the Department of Conservation (DOC) and community conservation groups to ensure the species has some hope of surviving on mainland New Zealand. The current management and recovery of brown kiwi relies heavily on a captive rearing programme, "Operation Nest Egg" (ONE), and over 200 kiwi are raised in this way each year (Colbourne et al., 2005; Holzapfel et al., 2008). The ONE programme involves removing wild kiwi eggs or young chicks from the nest, hatching any eggs in captivity, raising the chick to a specified age or weight (under either captive or semi-captive conditions) and releasing them back into the wild (Colbourne et al., 2005; Holzapfel et al., 2008). The purpose of ONE is to ensure the main threats to kiwi are mitigated by keeping them in captivity during their most vulnerable life stages (Bassett, 2012; Colbourne et al., 2005). Juveniles are typically released back in to the wild at approximately one kilogram of weight, usually around 4-6 months of age, at which time they are considered big enough to avoid being killed by their main predator, the stoat (*Mustela erminea*) (Bassett, 2012; Colbourne et al., 2005; JA. McLennan, Dew, Miles, Gillingham, & Waiwai, 2004). This captive rearing programme is an important tool for the conservation of kiwi and achieves significant outcomes such as raising the survival to adult-hood from 5% for wild-born kiwi to 65% for ONE kiwi (Colbourne et al., 2005). However this intensive rearing relies on housing immunologically-undeveloped juvenile kiwi at high density with high turnover rates, which may result in pathogen build up (Morgan, Alley, Pomroy, Castro, & Howe, 2012; Morgan et al., 2013; Morgan et al., 2014). One of the most prevalent and limiting diseases influencing the successful rearing of ONE kiwi is coccidiosis (Morgan et al., 2012), and coccidia have been described by Doneley (2006) as the most important gastrointestinal parasite of captive kiwi. Currently little information is available on many aspects of coccidiosis in kiwi (Morgan, 2013) making the management of this important disease a challenge for kiwi managers. Coccidiosis is estimated to cost the worldwide poultry industry $3 billion annually, and with this economical driver a vast array of research has been conducted on coccidia species affecting domestic chickens (*Gallus gallus*) (Dalloul & Lillehoj, 2006; McDougald, 2003; Peek & Landman, 2011). At present this information is extrapolated to kiwi in order to make management decisions (Morgan, 2013). However, the accuracy of this is unknown and it is vital that our understanding of both the biology of the coccidia species affecting kiwi, as well as the host-parasite interactions, are better understood.

An important interaction that occurs in many other avian coccidia infections is the variable shedding of coccidial oocysts throughout a 24-hour period, known as circadian variation in oocyst shedding. This phenomenon has been described in many different bird species for various species of coccidia (Boughton, 1933; Coelho et al., 2013; Lopez, Figuerola, & Soriguer, 2007; Martinaud, Billaudelle, & Moreau, 2009; Misof, 2004). When present, this variable oocyst shedding pattern limits the accuracy of parasite burden testing via oocyst counts on droppings and if it is not accounted for then the results of such testing are unreliable and may be misleading (Coelho et al., 2013; Filipiak, Mathieu, & Moreau, 2009; Misof, 2004). At present, it is unknown whether circadian variation of oocyst shedding occurs in kiwi, meaning management decisions based on oocyst counts on kiwi droppings may be flawed. The following thesis, and the research therein, seeks to investigate and define any oocyst shedding pattern that may occur in brown kiwi to allow better management of this iconic species. Further to this, the presence and prevalence of different *Eimeria* spp. at the research site is discussed, as is the use of toltrazuril to treat coccidiosis in kiwi.