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**Host-parasite interactions between  
*Plasmodium* species and New Zealand birds:  
prevalence, parasite load and pathology**

A thesis presented in partial fulfilment of the requirements for the degree of  
Master of Veterinary Science  
in  
Wildlife Health  
At Massey University, Palmerston North  
New Zealand

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## ABSTRACT

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Avian malaria, caused by *Plasmodium* spp., is an emerging disease in New Zealand and has been reported as a cause of morbidity and mortality in New Zealand bird populations. This research was initiated after *P. (Haemamoeba) relictum* lineage GRW4, a suspected highly pathogenic lineage of *Plasmodium* spp. was detected in a North Island robin of the Waimarino Forest in 2011. Using nested PCR (nPCR), the prevalence of *Plasmodium* lineages in the Waimarino Forest was evaluated by testing 222 birds of 14 bird species. *Plasmodium* sp. lineage LINN1, *P. (Huffia) elongatum* lineage GRW06 and *P. (Novyella) sp.* lineage SYATO5 were detected; *Plasmodium relictum* lineage GRW4 was not found. A real-time PCR (qPCR) protocol to quantify the level of parasitaemia of *Plasmodium* spp. in different bird species was trialled. The qPCR had a sensitivity and specificity of 96.7% and 98% respectively when compared to nPCR, and proved more sensitive in detecting low parasitaemias compared to the nPCR. The mean parasite load was significantly higher in introduced bird species compared to native and endemic species. The data suggests that introduced bird species such as blackbirds have a higher tolerance for *Plasmodium* spp. infections than endemic and native species. The high prevalence of infection and high parasite load in introduced passerines confirmed that they are important reservoirs for avian malaria in the New Zealand. A clinical case of avian malaria in a captive wildlife setting was described for a little penguin (*Eudyptula minor*) at Wellington Zoo. Nested PCR results and DNA sequencing confirmed infection of the deceased penguin with *Plasmodium (Huffia) elongatum* GRW06. A retrospective analysis of little penguin cases in the Massey University post mortem database, combined with nested PCR for *Plasmodium* spp. on stored liver tissue samples and DNA sequencing, revealed three additional mortality cases due to *P. elongatum* lineage GRW06, *P. relictum* lineage SGS1 and *P. sp.* lineage LINN1 in one captive and two wild little penguins. Our results suggest that avian malaria causes sporadic mortality in New Zealand's little penguins, but there is no evidence of mass mortality events due to avian malaria in this species.



## DECLARATION

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The studies presented in this thesis were completed by the author whilst a postgraduate student at 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.



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Danielle Sijbranda

Supervisor



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Brett Gartrell

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## **ETHICS APPROVAL AND CONSENTS**

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The handling of live birds during this research was approved by the Massey University Animal Ethics Committee (MUAEC protocol number 11/72), the New Zealand Department of Conservation (DOC Permit no. 34781-FAU) and the local land owners of the Waimarino Forest: Atihau-Whanganui Incorporation, Ngaporo-Waimarino Forest Trust, Pipiriki Incorporation and Ernslaw One Limited.



## THESIS STRUCTURE AND FORMAT

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This thesis comprises an introduction and literature review, three research chapters, and a general discussion chapter, followed by two sections containing references and appendices. The three research chapters were written as stand-alone papers with the intention of peer reviewed publication. Hence there is some replication in the introduction and discussion sections of these various chapters.

### *Chapter 1*

The “introduction and literature review” provides a framework of background information regarding *Plasmodium* parasites and their life cycle, the related pathogenic effects in birds, diagnostic techniques for avian malaria and examples of the impact of avian malaria infections during wildlife management situations.

### *Chapter 2*

“Avian malaria in introduced, native and endemic New Zealand bird species in a mixed ecosystem” describes a study evaluating the prevalence of various lineages of *Plasmodium* spp. in North Island robins and other bird species in the Waimarino Forest in order to evaluate this area as a source site for robin translocations. A modification of this chapter has been accepted as a research article by the New Zealand Journal of Ecology, and will be published in its 2016 edition 40(1). The article was first published on-line on the 3<sup>rd</sup> of September 2015 at <http://newzealandecology.org/nzje/3241>.

### *Chapter 3*

“Use of a real-time PCR to explore disease dynamics of avian malaria in a mixed New Zealand ecosystem” describes the development of a real-time PCR protocol for *Plasmodium* spp. in order to quantify parasite load in individual birds.

## ***Chapter 4***

“Mortality of little penguins (*Eudyptula minor*) in New Zealand due to avian malaria” describes a clinical case of avian malaria in a blue penguin in a wildlife rehabilitation setting at Wellington zoo, and incorporates findings of a retrospective review of little penguin post mortem cases from the Massey University post mortem database.

## ***Chapter 5***

The “general discussion” reflects on the findings and implications for New Zealand birds of the aforementioned studies, and discusses ideas for further research.

## ***References***

To prevent duplication of references, all references have been grouped together after Chapter 5.

## ***Appendices***

Research data and laboratory protocols for nested and real-time PCR are added as appendices for reference.

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