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

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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 galllinae* 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.

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Mirza Vaseem

Supervisor

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Brett Gartrell

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ACKNOWLEDGEMENTS

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.

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CHAPTER 1: LITERATURE REVIEW

1.1 New Zealand's raptors- a short description

The uniqueness of New Zealand as an island nation has led to the evolution of floral and faunal biodiversity that is found nowhere else in the world (Daugherty et al., 1993). About 200 species of birds occur in New Zealand today, of which more than 90 are endemic to the country, such as flightless birds like the kiwis *(Apteryx* spp) and wekas *(Galliralus* spp), parrots like the kaka *(Nestor meridionalis)* and the kea *(Nestor notabilis)* and several other species of passerines and seabirds (McLay, 1974) .

Living among the rich avifaunal biodiversity of New Zealand are four species of raptors, including two endemic species the New Zealand falcon *(Falco novaeseelandiae)* and the morepork *(Ninox novaeseelandiae)*, the self-introduced Australasian harrier *(Circus approximans)* and the deliberately introduced little owl *(Athene noctua)* (Higgins, 1999). These birds occupy an important scavenging and predatory niche in the country's ecosystem, since New Zealand previously lacked mammalian predators (Kross et al., 2013) However, following human colonization of New Zealand, both endemic and introduced raptors faced severe threats of persecution, loss of prey base and habitat alteration, that led to the extinction of several raptor species, the Haast eagle *(Harpagornis moorei),* Eyle's harrier *(Circus eylesi)* and the laughing owl *(Sceloglaux albifacies),* and continues to threaten the existence of the country's only endemic falcon, the New Zealand falcon today (Hume & Walters, 2012). The following sections provide a brief description of New Zealand's raptor species in the three avian families- Falconidae, Accipitridae and Strigidae.

1.2 New Zealand falcon (*Falco novaeseelandiae*)

New Zealand falcon is New Zealand's only endemic falcon species that was first reported by Sir Joseph Banks in 1790 (Fox, 1977). Threats of persecution, introduced predators and habitat degradation led to significant decline in falcon numbers following European colonization, but after being identified as a protected species in 1970, their population size is now stable at 3000-4500 pairs (Hitchmough et al, 2007; Fox, 1977). These falcons are currently classified as 'Nationally Vulnerable/ Nationally Endangered' by the New Zealand threat

classification system and as 'Near Threatened' by the IUCN (Hitchmough et al., 2007).

1.2.1 Distribution and Biology

A member of the family Falconidae, the New Zealand falcon is a medium-sized raptor that occupies large territories in the eastern parts of the North Island and almost the whole of South Island (Higgins, 1999). The birds have been recorded to show territorial behaviour and occupy large spaces of 25-75 km² (Higgins, 1999). Though no subspecies among NZ falcons have been recorded so far, Fox in 1988 described three forms- bush, Eastern and Southern based on size, ecology, plumage and geographical distribution (Fox, 1977). The Bush form can be found inhabiting the eastern parts of the NI, while the Eastern form mainly occupies the east coast of the SI. The Southern form however, is a coastal variant that inhabits Fiordland, Stewart Islands and Auckland Islands (Higgins, 1999). New Zealand falcons have also been found to adapt and survive with longer breeding seasons in pine plantations in the North Island (Seaton et al., 2009). Additionally, projects involving introduction of falcons in vineyards to curb damage to grapes caused by passerines has also shown impressive prospects (Kross et al., 2012).

Of the three forms, the Eastern NZ falcon is the largest in size and palest in colour (Higgins, 1999). But the Southern and bush falcons are more or less similar in size and plumage colouration (Higgins, 1999). Like most raptors, females are larger than males and can be easily distinguishable due to their darker plumage than the males (Higgins, 1999).

1.2.2 Breeding

The breeding season of New Zealand falcons has been stated to extend from late September until early February, though most egg laying ceases after November (Higgins, 1999) (personal communication, Andrew Thomas, 2014). The falcons cohabit in simple pairs throughout the breeding season with elaborate courtship rituals of chasing and food sharing followed by mating (Fox, 1977). The falcons reach sexual maturity after 20 months, though there has been a single report of a female attaining maturity at 11 months of age (Seaton & Hyde, 2008). The females may lay 2-4 eggs in scrapes on the ground. The lack of predators until the early 1800 has allowed the falcons to forego the practice of building safe nests in trees or on cliff edges, however, this practice leaves the adults and chicks vulnerable to attacks after the introduction of rodents and cats (Kross et al., 2013; Fox, 1977). Following an incubation period of 29-35 days, the chicks hatch in an altricial state and are provided food and shelter by both parents, though feeding is carried out solely by the female (Higgins, 1999). The nestlings remain close to the scrape until 32 days of age and by 52 days, they are able to fly and even hunt for food (Higgins, 1999). Although, the parents still continue to provide food to their young ones through ariel passes (Higgins, 1999).

1.2.3 Diet and feeding habit

New Zealand falcons feed on a wide variety of birds, small mammals and reptiles. Kross et al (2012) identified the most frequently hunted prey included chaffinches *(Fringilla coelebs)*, warblers *(Gerygone spp.)*, tomtits *(Petroica macrocephala)*, tuis *(Prosthemadera novaeseelandiae)*, blackbirds *(Turdus merula)*, rodents, insects and skinks. The falcons are ambush and attacking predators and have rarely been observed scavenging or stashing carrion for later feeding (Hitchmough et al., 2007). Although the diet of New Zealand falcons mostly includes small passerines, they are capable of hunting larger prey like hares *(Lepus europaeus)* and ring-necked pheasants *(Phasianus colchicus)* that are about three to six times their own body weight (Hyde & Seaton, 2008; Fox, 1977). The diet of New Zealand southern falcons living on Auckland Islands is significantly different from that of their mainland counterparts and comprises more of seabirds like petrels *(Hydrobatidae spp.)* and Antarctic prions *(Pachyptila desolata)* than land-birds (Hyde & Worthy, 2010).

1.3 Australasian harrier (*Circus approximans*)

The Australasian harrier or the swamp harrier is New Zealand's largest raptor species that was self-introduced in the late 1800s due to changes in the landscape of the country following European colonisation (Higgins, 1999). Australasian harrier populations in New Zealand are stable and do not face a decline in the near future, but the birds do face several threats on a daily basis. Many harriers die from vehicular trauma while scavenging on road kills, 1080 and lead poisoning following the consumption of contaminated prey (Higgins, 1999).

1.3.1 Distribution and Biology

Unlike their Australian counterparts that are mostly confined to wetlands and swamps, the Australasian harriers in New Zealand occupy large tracts of open farmland where live prey and carrion are abundant (Eakle, 2008; Higgins, 1999). Apart from farmland, they may also be found along river valleys, tussock grasslands and scrub vegetation (Higgins, 1999). A survey of Australasian harriers across New Zealand by Eakle in 2008 revealed that these birds were thriving in abundance on both the islands, with a population estimate of at least 1 harrier for every 16.9 kilometres travelled (Eakle, 2008). The increased availability of both live prey and carrion in the form of passerines, rodents and discarded dead lambs in forested regions and farmlands respectively could be one of the reasons for the harrier's vast geographical distribution across the country (Higgins, 1999).

Australasian harriers are similar in size to large kites (*Milvus spp.*) and adult females can weigh close to 900 grams, though males are smaller in comparison,

with a body weight of about 650 grams (Hitchmough et al., 2007). Both sexes are similar in appearance with respect to plumage colouration, except for the sub-terminal band on the under-wing, which is broader in females compared to males (Higgins, 1999). Plumage colour changes from dark brown in young harriers to a paler version in adults (Higgins, 1999).

Additionally, the birds observe a migratory behaviour in Australia, but this practice seems to be rare among the New Zealand species (Higgins, 1999). Only a few accounts of local migration during the non-breeding season, possibly governed by the abundance of food have been reported (Higgins, 1999).

1.3.2 Breeding

The breeding season falls during July-August and is characterised by aerial displays, food sharing and courtship, followed by mating (Higgins, 1999). The nesting behaviour of swamp harriers is not well-understood, but they have been found to lay eggs in crops, which are usually damaged by machinery, trampled by sheep or predated upon by rodents (Higgins, 1999). The female lays about 1-7 eggs and the young ones fledge during the month of December, during which time they become independent (Higgins, 1999). The breeding population of harriers in New Zealand appears to be secure and has been calculated at 1 breeding pair/120 ha (Higgins, 1999).

1.3.3 Diet and feeding habit

The main prey items of the harrier are rats, hares, rabbits, birds, insects and reptiles, although scavenging road-kills also forms a significant portion of their diet (Higgins, 1999). On the mainland, remains of adult hares, hedgehogs *(Erinaceus europaeus)*, magpies *(Gymnorhina spp.)*, grasshoppers, crickets, ducks *(Anas spp.)*, skylarks *(Alauda arvensis)*, song thrushes *(Turdud philomelos)*, tomtits, goldfinches *(Carduelis tristis)*, sparrows *(Passer domesticus)* and yellowhammers *(Emberiza citrinella)*, among several other species have been recovered from nests and gut contents (Higgins, 1999), while harriers dwelling on islands were found to be feeding on seabirds such as fairy prions *(Pachyptila turtur)* and blue penguins *(Eudyptula minor)* (Hawke et al., 2005)*.* Events of harriers preying on chicks of native ground-nesting birds have been reported, but whether it contributes to their decline over the years is yet to be ascertained (Sanders & Maloney, 2002).

1.4 Morepork (*Ninox novaeseelandiea*)

Moreporks are small, earless owls and New Zealand's only existing native owl species (Heather & Robertson, 1996). Three sub-species have been identified so far, *N. novaeseelandiae* from New Zealand, *N.n. albaria* from the Lord Howe

Island, which has gone extinct and the critically endangered *N.n. undulate* that are found on the Norfolk Islands (Heather & Robertson, 1996; Olsen, 1996).

1.4.1 Distribution and Biology

Moreporks are widely distributed and abundant in New Zealand on both the North Island and the South Island (Heather & Robertson, 1996). They also reside in the surrounding islands like the Kapiti, Three kings, Little and Great Barrier, Stewart Island and the Norfolk Island, although in far lesser numbers than the New Zealand population, (Heather & Robertson, 1996). They inhabit forests, farmlands and urban areas, conveniently adapting to their respective environment (Heather & Robertson, 1996).

Plumage in adults is variable but all birds are predominantly chocolate brown in colour with whitish brows and spotting on the head, neck and mante regions (Heather & Robertson, 1996). The underparts are brownish white with darker chocoloate brown streaks. Juvenile birds contain small areas of white down feathers but soon turn smoky-brown within 5 weeks time (Heather & Robertson, 1996).

1.4.2 Breeding

The mating season usually begins in September and is followed by egg-laying in October which continues until the end of November (Stephenson, 2006; Heather & Robertson, 1996). The female lays 1-2 eggs and solely incubates them for a period 0f 30 days, while the male gathers food for the female and nestlings (Stephenson, 2006; Heather & Robertson, 1996). The chicks fledge after a period of 35 days but the parents remain close to the fledglings (approximately 1 metre), providing food and protection until the young ones disperse and expand their territory after about 2-3 months following fledging (Stephenson, 2006).

The nest sites are simple and have been recorded in tree hollows, abandoned sparrow nests, dense vegetation, tree forks and even nest boxes (Heather & Robertson, 1996). They have small entrances of less than 20 cm and are usually situated at a height of over 2 metres above the ground (Heather & Robertson, 1996). Moreporks do not favour any particular type of nest site and may choose any appropriate hollow to lay eggs. Chick mortalities are common due to siblicides or predation by rodents, cats and even harriers (Heather & Robertson, 1996). Stephenson in 2006 theorised that the breeding success of moreporks on Mokoia Islands had reduced following eradication of rats, but suggests further research to understand the effects of eradication procedures on breeding success of moreporks.

1.4.3 Diet and feeding habit

Insects and arachnids are the most predominant prey items consumed by the Moreporks. Spiders, wetas, moths, grasshoppers and beetles have been regularly found in the nests and guts of these birds (Stephenson, 2006; Heather & Robertson, 1996). Apart from these, they also prey on passerines like sparrows and finches, lizards and small mammals like rats and mice (Stephenson, 2006; Heather & Robertson, 1996). The moreporks inhabiting off-shore islands also take in short-tailed bats and petrels as prey (Stephenson, 2006; Heather & Robertson, 1996). There have been reports of moreporks preying on the nationally vulnerable long-tailed bat *(Chalinolobus tuberculatus)*, but the impact of predation by moreporks on long-tailed bats is yet to be ascertained (Borkin & Ludlow, 2009).

1.5 Diseases and threats

New Zealand's raptors currently face threats such as predation of eggs and brooding females by cats or rodents, secondary poisoning from the consumption of lead or 1080, deforestation, vehicular trauma and in case of moreporks, competition for survival by introduced species like the little owls (Butchart, 2014; McLelland et al., 2011; Higgins, 1999; Stephenson et al., 1999; Heather & Robertson, 1996). It is believed that the extinction of moreporks from the Lord Howe Islands in the 1950s occurred following the introduction of the Southern boobook (*Ninox boobook*) and egg-predation by rodents (Heather & Robertson, 1996). Australasian harriers are still subjected to persecution under the belief that they damage property and kill livestock in spite of being classified as partially protected by the government under Schedule 2 and being recognized as a species that keep the rodent population at check, along with aiding in carcass disposal by feeding on carrion (Gee, 2006).

However, the only infectious disease reported among New Zealand's raptors so far, is an incidental finding of serratospiculosis in an adult male falcon suffering from a traumatic fracture of the left wing (Green et al., 2006). Radiographic examination of the bird revealed the presence of opaque masses in the caudal abdominal air sacs and progressively worsening respiratory signs that led to its death shortly after admission. Necropsy confirmed the presence of several nematodes belonging to the species *Serratospiculum guttatum* in the air sacs (Green et al., 2006).

Evidently, information regarding infectious diseases capable of affecting New Zealand's raptors is scarce and further research is required to determine the effect of disease causing pathogens on New Zealand's raptors.

1.6 Diseases of Falconiformes and Strigiformes

Very little information regarding the major causes of morbidity and mortality in New Zealand's three raptor species is available in literature, especially in terms of diseases. Hence, a comprehensive review of common parasitic, bacterial, viral and fungal diseases of falconiformes and strigiformes in different parts of the world has been described below to understand the susceptibility of raptors to various pathogens. Our study focuses only on infectious diseases that might be capable of affecting New Zealand's raptors, Hence the following review of literature focuses only on the various infectious causes of disease encountered in global raptor populations. This review can serve as a reference list for the different kinds of pathogens that can infect raptors in New Zealand and also serve as a source of differential diagnoses for the same.

1.6.1 Protozoa

Coccidia species have frequently been isolated from both wild and captive raptors, either as an incidental finding or as an infectious cause of mortality, with higher number of infections observed in captive birds than wild (Cooper, 2002). The most commonly encountered coccidia species in raptors, belong to the genera *Caryospora*, *Cryptosporidium*, *Sarcocystis* and *Eimeria* and have been identified in accipiters (Table 1), falcons and strigiformes (Cooper, 2002; M. G. Papazahariadou, et al, 2001; Baker, et al, 1996; Upton, et al, 1990). Pathogenicity of these protozoans in raptors is debatable, since the birds regularly shed oocysts in their mutes without showing any apparent signs of illness (Mueller et al, 2008; Forbes & Simpson, 1997). However, severe debilitating symptoms such as diarrhoea, progressive weight loss, anorexia and respiratory distress have been documented among birds of prey that were found to carry high loads of coccidia within their systems (Mueller et al., 2008). Raptors can serve as either intermediate or definitive hosts to these parasites, although it is unclear whether the oocysts are transmitted through consumption of infected prey, through environmental contamination or both (Forbes & Fox, 2005; Forbes & Simpson, 1997).

a) Caryospora spp

Caryospora spp appear to be the most frequently encountered protozoan parasite with over 7 species being identified in accipiters alone and two new species being discovered in 1999 (Table 2) (Volf et al, 2000). In addition, the prevalence of *Caryospora* organisms in Strigiformes is much lower in comparison to Falconiformes and only four species of caryospora have been identified in strigidae so far- *C. henryae, C. bubonis, C. neofalconis* and *C. strigis* (Papazahariadou et al., 2001; Cawthorn & Stockdale, 1982).

Caryospora infections usually occur in chicks or juvenile birds and are characterized by haemorrhagic-diarrhoea, regurgitation, anorexia, progressive loss of body condition and muscle spasms towards the terminal stages of the disease (Forbes & Simpson, 1997). However, in most cases the birds may not display any signs of disease before succumbing to the infection (Forbes & Simpson, 1997). It has been postulated that, young birds of less than 55 days of age are more susceptible to the disease due to the lack of active immunity within their system (Forbes & Fox, 2005; Forbes & Simpson, 1997). Therefore, scientists are currently working on developing vaccines against *C. neofalconis, C. megafalconis, C. henryae and C. kutzeri* in order to assist the young birds in combating the infection. However, efficacy of these vaccines is still unknown (Forbes & Fox, 2005).

Table 1:Various coccidia spp encountered in Accipitridae species

Coccidia species		Accipitridae host	References
Caryospora spp	*C. henryae*	Black kite (*Milvus migrans*)	Redig, 1993
	C. arcaye	Road-side hawk (*Buteo magnirostris*),	Volf et al., 2000
		Broad-winged hawk (*Buteo platypterus*),	
	C. kansasensis	Swainson's hawk (*Buteo swainsonii*),	Volf et al., 2000
	C. lindsayi	Red-tailed hawk (*Buteo jamaicensis*)	Volf et al., 2000
	C. uptoni	Red-tailed hawk (*Buteo jamaicensis borealis*)	Volf et al., 2000
Eimeria spp	*E. accipitris*	European sparrowhawk (*Accipiter nisus*)	Redig, 1993
		Booted eagle (*Hieraaetus pennatus*)	
	E. asturi	Goshawk (*Accipiter gentilis*)	Redig, 1993
	E. glareoli	Buzzard (*Buteo buteo*)	Redig, 1993
Frankelia spp	*F. microti*	Buzzard (*Buteo buteo*),	Redig, 1993
		Red-tailed hawk (*Buteo jamaicensis*)	
Cryptosporidium spp		Common buzzard (*Buteo buteo*)	Papazahariadou et al., 2008

Table 2: *Caryospora species encountered in various Falconidae species*

Caryospora species	Host	References
C. neofalconis	Lanner falcon (*F. biarmicus*), Prairie falcon (*F. mexicanus*), Peregrine falcon (*F. perigrinus*), Eurasian hobby falcon (*F. subbuteo*), Common kestrel (*F. tinnunculus*), Merlin (*F. columbarius*)	Forbes & Fox, 2005 Forbes & Simpson, 1997 Upton et al., 1990
C. falconis	Peregrine falcon (*F. perigrinus*), Eurasian hobby falcon (*F.subbuteo*, Common kestrel (*F.tinnunculus*)	Upton et al., 1990
C. kutzeri	Laggar falcon (*F. juggar*), Saker falcon (*F. cherrughi*), Gyrfalcon (*F. rusticolus*), Lanner falcon (*F. biarmicus*), Prairie falcon (*F. mexicanus*), Peregrine falcon (*F. perigrinus*), Common kestrel (*F. tinnunculus*), Eurasian hobby falcon (*F. subbuteo*)	Upton et al., 1990
C. cherrughi	Saker falcon (*F. cherrughi*)	Alfaleh et al, 2013
C. biarmicus	Lanner falcon (*F. biarmicus*)	Alfaleh et al., 2013
C. boeri	Common kestrel (*F. tinnunculus*)	Alfaleh et al., 2013
C. henryae	Common kestrel (*F. tinnunculus*), Eurasian hobby falcon (*F.subbuteo*)	Alfaleh et al., 2013
C. megafalconis	Saker falcon (*F. cherrughi*), Gyrfalcon (*F. rusticolus*), Common kestrel (*F. tinnunculus*)	Alfaleh et al., 2013

b) Cryptosporidium spp

A highly pathogenic, zoonotic disease of birds, cryptosporidiosis is caused by any of the three species of Crysptosporidium namely, *C. baylei, C. meleagridis* and *C. galli* (Molina-Lopez et al., 2010). The Apicomplexan protozoans have a wide host range and possess a high prevalence among both captive and free-living birds (Molina-Lopez et al., 2010). Though frequently reported in poutry birds, not much information is available regarding its prevalence and pathogenicity in Strigiformes and Falconiformes. Cases have been reported from a captive Gyrfalcon *(Falco rusticolus)* that suffered from *C. parvum* infection, Scop's owls *(Otus scops)* in Spain and Striped owls *(Pseudoscops clamator)*, barn owls *(Tyto alba)* and great horned owls *(Bubo virginianus)* from South America and transmission of the disease is by direct contact with contaminated ocular and nasal discharges, mutes and fomites (da Silva et al., 2009; Plutzer & Karanis, 2009; van Zeeland et al, 2008). Furthermore, the possibility of an outbreak is higher in captive birds due to higher densities and restricted habitats compared to wild populations, and increased contact with other species and humans which can increase the risk of disease transmission, particularly where husbandry practices are poor (da Silva et al., 2009).

Unlike typical coccidiosis, which is characterized by gastrointestinal symptoms in mammals, cryptosporidiosis in birds causes respiratory distress that can escalate to dyspnoea, epiglottal swelling, tracheitis, rhinitis, conjunctivitis and in one report, otitis media in a Saker falcon (*Falco cherrughi)* (Bougiouklis et al. 2013; Molina-Lopez et al., 2010). These clinical signs occur as a result of the sporozoites penetrating the respiratory epithelium of the host, wherein the pathogen completes the remainder of its life cycle (Sréter, 1998).

c) Trichomonias spp

Trichomoniasis is caused by a flagellate protozoan called *Trichomonas gallinae* and has been found to affect several species of columbiformes, anseriformes, falconiformes and strigiformes (Atkinson et al, 2008). The organism has a worldwide distribution except in cold regions such as Antarctica, Greenland, Alaska, northern Europe and Asia (Atkinson et al., 2008). The disease is pathogenic to raptors, capable of causing significant mortalities in the affected birds and has been reported from great horned owls, Eurasian eagle owl *(Bubo bubo)*, European scops owl, tawny owls *(Strix aluco)*, sakers, peregrines, lanners *(Falco biarmicus)* and gyrfalcons *(F. rusticolus)*, goshawks *(Accipiter gentilis)*, bald eagles *(Haliaeetus leucocephalus)* and Cooper's hawks *(Accipiter cooperii)* among many others (Atkinson et al., 2008; Samour & Naldo, 2003; Cooper & Petty, 1988)

Infected birds appear weak and suffer from dysphagia, neurological-signs and develop whitish-yellow plaques or nodules in the oropharynx, oesophagus, nasal sinus and buccal cavity (Samour & Naldo, 2003). The disease may also prevail in

conjunction with other concomitant diseases like, aspergillosis and pseudomonas infections (Tarello, 2011; Samour & Naldo, 2003). Diagnosis of the disease is done by direct observation of the protozoan under a microscope following wet-mount preaparation of oral swab or a crop wash (Amin et al., 2014).

d) Toxoplasma

Toxoplasms gondii is a protozoan parasite with worldwide distribution and has been found to infect all worm-blooded species (Cabezon et al., 2011). The organism follows an oro-faecal route of transmission and utilises felids as its definitive hosts that shed oocysts in their faeces for a prolonged duration of time following infection (Cabezon et al., 2011). However, several species of mammals and birds have been found to play the role of intermediate hosts in the transmission of this disease by ingesting tissues contaminated with *T. gondii* cysts that contain bradyzoites of the organism (Cabezon et al., 2011).

The prevalence of *T. gondii* is significantly high in various species of Columbiformes, Passeriformes and Falconiformes, but the susceptibility for disease varies between species (Dubey, 2002). Raptors are suspected to be infected by eating the flesh of intermediate hosts such as small birds and mammals rather than by direct ingestion of oocysts. This finding compliments the observation made by Cabezon et al, (2011) who believed that *T. gondii* was more prevalent in carnivorous and scavenging birds. Since pigeons and small mammals are primary sources of prey for accipiters and the birds also scavenge opportunistically, it puts them at a higher risk of contracting the disease (Cabezon et al., 2011). Studies aimed at determining the prevalence of *T. gondii* in accipiters found high antibody titers for the disease among the common buzzard *(Buteo buteo),* Eurasian sparrowhawk *(Accipiter nisus),* black kite *(Milvus migrans),* short-toed eagle *(Circaetus gallicus)* and Northern goshawk *(Accipiter gentilis)* in Spain and Portugal (Cabezon et al., 2011; Lopes et al., 2011). High antibody titers for *T. gondii* were also observed in red-shouldered hawks *(Buteo lineatus),* red-tailed hawks *(Buteo jamaicensis),* Cooper's hawks *(Accipiter cooperii),* golden eagles *(Aquila chrysaetos)* and bald eagles *(Haliaeetus leucocephalus*) in the USA (Lindsay et al., 1991; Szabo et al., 2004). But it is unclear whether the infected birds exhibited clinical signs of the disease or were merely asymptomatic carriers since Dubey (2002), suggests that Accipitriformes rarely suffer from clinical toxoplasmosis in spite of possessing high antibody titers for the protozoan. So much so, that even experimental feeding of *T. gondii* infected tissues failed to elicit any clinical signs of disease in the birds (Dubey, 2002).

1.6.2 Haemoprotozoa

a) Plasmodium spp.

Avian malaria is caused by *Plasmodium* spp., an intra-cellular haemoprotozoan belonging to the phylum Apicomplexa (Remple, 2004). Ten species of *Plasmodium* have been isolated from birds of prey so far, of which only *P. relictum, P. circumflexum and P. cathemerium* are known to be pathogenic (Remple, 2004). The first reports of the disease in accipiters were in brahminy kites *(Haliastur indicus)*, white-eyed buzzards *(Butastur teesa)*, bald eagles and several species of hawks from Europe and North America (Remple, 2004). Subsequently, the parasite has been isolated from buzzards *(Buteo* spp) and black kite *(Milvus migrans)* in Iraq, from Eurasian sparrowhawks *(A. nisus)* in Japan, Northern goshawk *(A.gentilis),* common buzzard *(Buteo buteo),*rough-legged buzzard *(B. lagopus),* Western marsh harrier *(Circus aeruginosus)* in Europe and a shikra *(A. badius)*in Thailand (Perez-Rodriguez et al., 2013)In falcons, the first case of avian malaria was reported in a gyrfalcon in 1956 in Alaska (Remple, 2004). By the end of 1972, mortalities were reported from American kestrels (*F. sparverius*), European kestrels (*F. tinnunculus*) and grey falcons (*F. hypoleucos*). Though *Plasmodium spp.,* have been isolated from several species of owls like spotted owls *(Strix occidentalis)*, eagle owls, barn owls and tawny owls from Europe and North America, not many reports of clinical infections have been documented in Strigiformes (Ortego & Cordero, 2009; Ishak et al., 2008; Krone et al., 2001).

Plasmodium spp. are spread between birds through mechanical vectors like Anopheles and Culicine mosquitoes (Remple, 2004). Once the sporozoites are inoculated in a healthy host, they undergo rapid multiplication to produce merozoites. These, upon further multiplication and several stages of merogony and gametogony, produce gametocytes that are picked up by the vectors. The gametocytes develop in to sporozoites within the mosquitoes and are transmitted into healthy hosts to complete the life-cycle (Valkiunas et al., 2008). The protozoan mainly affects the reticulo-endothelial system and the infected birds may show signs of anemia, anorexia, lethargy, hepatomegaly, splenomegaly biliverdinurea and death (Valkiunas et al., 2008).

b) Haemoproteus spp

Haemoproteus spp are intracellular parasites that infect the erythrocytes of birds, reptiles and mammals (Valkiunas et al., 2008). They are globally distributed and organisms like *H. nocturnae, H. syrnii, H. ilanpapernai* to name a few, have been identified in several species of raptors like Phillipine scops owl *(Otus megalotis)*, Phillipine eagle owls *(Bubo philipensis)*, brown hawk owls *(Ninox scutulata)*, snowy owls *(Bubo scandiacus)*, spotted wood owls *(Strix seloputo)*, tawny owls, collared owlets *(Glaucidium brodiei)*, red-tailed hawks *(B. jamaicencis)*, red-shouldered hawks *(B. lineatus)*, Cooper's hawks, Western

marsh harriers *(C. aeruginoses)*, European kestrels *(Falco tinnunculus)*, Eurasian hobbies *(Falco subbuteo),* American kestrels *(Falco sparverius)* from North America, South America, Europe and Asia (Bandoy, 2009; Karadjian et al 2014; Krone et al., 2001; Apanius & Kirkpatrick, 1988)

The protozoan requires mechanical vectors like Hippoboscid flies for its transmission and no direct horizontal or vertical transmission is possible (Johnson, 2009). Clinical signs in affected birds are similar to that of *Plasmodium* spp infections, where in the birds suffer from anemia, weakness, emaciation, hepatomegaly, splenomegaly and death (Johnson, 2009; Remple, 2004). Though largely asymptomatic, the disease may flare up as a result of stress and the presence of a prevailing primary infection that may have rendered the bird immune-compromised (Remple, 2004).

c) Leucocytozoon spp

Leucocytozoon spp. are globally distributed haemoprotozoans and have been reported from several species of Falconiformes and Strigiformes like peregrine falcons *(Falco peregrinus)*, common kestrels *(Falco tinnunculus)*, Eleonora's falcon *(Falco eleonorae)* red-shouldered hawks, red-tailed hawks, common buzzards, Cooper's hawks and bald eagles, tawny owls, Sjostedt's owls *(Glaucidium sjostedti)*, barred owls *(Strix varia)*, spotted owls, African wood owl *(Strix woodfordii)*, barn owls, little owls and eagle owls from the Americas, Europe, Africa and Asia (Karadjian et al., 2014; Tarello, 2008a; Krone et al., 2001; Remple, 2004; Cooper, 2002)

About 60 species of *Leucocytozoon* have been identified so far but only *L. todii* in Falconiformes and *L. ziemanni* or *L. danilewsky* in Strigiformes has been found to cause clinical leucocytoozonosis (Johnson, 2009; Remple, 2004). The disease is transmitted through the bite of mechanical vectors, like Simuliid flies and the organism possesses a life-cycle similar to that of Haemoproteus, except that in the former, gametocytes are produced only in the leucocytes and erythrocytes (Remple, 2004). Though apathogenic, compromised immunity and stress may render adult birds at a higher risk of contracting the disease (Johnson, 2009). The affected birds, especially young ones may show signs of weight loss, anemia and anorexia before succumbing to the disease (Remple, 2004). Autopsy may reveal the presence of gametocytes in blood cells and megaloschizonts in organs like the brain, liver, intestines, kidneys and lungs (Remple, 2004).

d) Babesia spp

Babesiosis is a frequently encountered disease in Falconidae, but seems to be rare in Accipitridae and Strigidae (Beaufrere et al 2007; Remple, 2004). Two species of *Babesia, B.shortii* and *B.moshkovskii* have been found to cause clinical signs of disease in raptors, of which the former usually encountered in falcons and the latter in accipiters (Ghazaei, 2007). A handful of babesiosis cases in

accipiters have been reported from North America, Europe and the Middle East in bearded vultures *(Gypaetus barbatus),* griffon vultures *(Gyps fulvus)* and a Steppe Eagle (*Aquila rapax)* (Tarello, 2005). Among Falconidae species, saker falcons *(Falco cherrug)*, peregrine falcons *(Falco peregrinus)* and gyrfalcons *(Falco rusticolus)* in Kuwait and Dubai were diagnosed with babesiosis (Remple, 2004). Mortalities in European kestrels *(Falco tinnunculus)* and Prairie falcons *(Falco mexicanus)* have also been reported from Europe and the Americas, indicating a global prevalence of both, the pathogen and its vector amongst falcon populations worldwide (Croft & Kingston, 1975). However, only two cases have been reported in a great horned owl and a barn owl so far from the USA and Northern Africa respectively (Remple, 2004).

The organism is highly pathogenic and is usually accompanied by concomitant infections like bumblefoot, aspergillosis and serratospiculosis (Tarello, 2008a). Furthermore, pathogenesis of babesiosis depends on the local population of Ixodid ticks in the area for its transmission, since direct contact between infected and healthy birds is not required for the spread of disease (Croft & Kingston, 1975). Babesiosis is characterized by lethargy, hypochromic anaemia, loss of body weight, blood in mutes[1] and hepatitis, although it is difficult to diagnose the disease solely based on clinical signs, since it is usually accompanied by a mixed infection (Tarello, 2006a). In spite of its notable prevalence, babesiosis in raptors is not well documented and treatment is still dependant on clinical trials, though results have been promising so far.

1.6.3 Nematodes
Several nematode species have been identified in Europe, America and Asia. Most nematodes possess a unique life-cycle and do not readily cause disease unless the host is immune-compromised or parasite numbers are extremely high, in which case, the endoparasites take an upper hand and cause a debilitating infection (Cooper, 2002). Nematodes are ubiquitous in their existence among raptor populations, although some may be more pathogenic than the others.

a) Serratospiculum spp
 Serratospiculum spp. are filarial nematodes which are distributed all over the world and capable of causing debilitating disease in carnivorous birds (Caliendo & McKinney, 2013; Atkinson et al., 2008). The nematode is more common in falconiformes, but has also been associated with mortalities in accipiters, particularly from North America (Ackerman et al, 1992). In many cases, *Serratospiculum* spp are usually present as incidental parasites, but may cause mortality due to an excessive worm load or when the bird is suffering from a concomitant infection (Caliendo & McKinney, 2013).

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[1] Commonly used term that refers to the combined faecal and urate droppings of raptors

The parasite possesses an indirect life-cycle and requires an intermediate host, usually insects like beetles, grasshoppers and wood lice for the propagation of the disease (Johnson, 2009; Atkinson et al., 2008). When a healthy bird ingests an infected beetle, the larval parasites exit the proventriculus and colonise in the air sacs before developing into adults and migrating to the lungs where they are coughed up, re-ingested and passed through the gastrointestinal tract (Atkinson et al., 2008). The affected birds may show severe air-sacculitis, emaciation, dyspnoea, loss of body condition and can also accompany a secondary bacterial or fungal infection, following which the bird succumbs to the infection (Caliendo & McKinney, 2013). Though capable of causing mortality in raptors, no wide-spread disease out-break has been reported so far in raptor populations (Atkinson et al., 2008).

b) Capillaria spp

It is a common parasite affecting both free-living and captive raptor populations belong to the genus *Capillaria*. These nematodes have been recorded in red-tailed hawks, common buzzards, great horned owls, Western screech owls, burrowing owls, scop's owls, tawny owls, little owls, Eurasian eagle owls from all over the world (Ferrer et al 2004; Johnson, 2009; Baker et al., 1996). *Capillaria* spp*. observe a direct life-cycle and the disease may either spread through direct contact or the parasite may utilise earthworms as paratenic hosts and birds that frequently feed on earthworms are especially prone to contracting the infection (Johnson, 2009).

It has been observed that the adult worms localise in the oropharynx and intestine of the affected birds and cause white, plaque like lesions in the buccal cavity, which get exacerbated by secondary bacterial infections (Johnson, 2009). The disease is usually asymptomatic, but when the birds are immune-suppressed, clinical signs like diarrhea, listlessness and emaciation may be seen and the birds die of starvation have been observed in accipiters and owls (Johnson, 2009). In falcons, three species of *Capillaria* have been identified so far, *Eucoleus sp., Capillaria tennuissima* and *Baruscapillarisa falconis* and the disease is characterized by necrosis and haemorrhage in the buccal cavity, regurgitation, anorexia, blood in mutes, convulsion and cachexia (Tarello, 2008b). However, in spite of its frequent occurrence, not much information regarding the prevalence and pathogenicity of the parasite is available.

c) Cyathostomes and Syngamus infections

Nematodes belonging to the *Cyathostoma* and *Syngamus* are relatively common in raptors and mount an infection only when the birds are immune-compromised (Smith, 1996). These nematodes possess a direct life cycle and observe direct transmission between infected birds or ingestion of contaminated prey (Smith, 1996). Clinical signs can range from weight loss and anorexia and

an excessive worm load may cause death due to intestinal obstruction (Smith, 1996).

Cyathostoma and *Syngamus spp.* colonise in the upper respiratory tract and cause respiratory distress due to inflammation or obstruction of the airway (Smith, 1996). The affected birds may show gasping, open-mouthed breathing and severe signs of dyspnoea (Smith, 1996). These nematodes are usually encountered as incidental findings when their hosts are immune-suppressed or experience a heavy worm load and have not caused significant disease outbreaks so far.

1.6.4 Trematodes, Cetodes and Acanthocephalans

Diplostomatid species are the most commonly encountered trematodes in raptors, but are usually asymptomatic and rarely cause severe disease, although a single mortality due to the parasite has been recorded in a bald eagle (Johnson, 2009; Smith, 1996). Similarly, cestodes like *Taenia, Strigea, Cladotenia, Choanotaenia* are relatively non-pathogenic parasites that localise in the small intestine of raptors and may cause weight loss and diarrhoea if the worm load is higher than normal (Johnson, 2009; Smith, 1996). Though these parasites possess a high prevalence, their pathogenicity is low and hence may be considered as less of a risk to accipiters.

'Thorny-headed worms' or Acanthocephalans have been commonly reported from Falconiformes and Strigiformes; but are considered to be rare and mildly pathogenic unless the host is severely immune-compromised or is harbouring a heavy burden of the parasite (Johnson, 2009; Borgsteede et al., 2003; Smith, 1996). These organisms colonise in the intestines following ingestion of infected intermediate-hosts like insects or small mammals and are capable of penetrating the enteric mucosa to cause peritonitis in infected birds. The disease is characterized by severe diarrhoea, weight loss, depression and anorexia. But infected birds respond positively to treatment and risk of mortality is usually low (Tarello, 2009).

1.6.5 Bacteria

a) Mycobacterium avium

Avian tuberculosis is a debilitating disease of raptors caused by the bacteria *Mycobacterium avium,* also referred to as the *Mycobacterium avium* complex, which includes other *Mycobacterium* species like *M. intracellulare* and *M. genavense* (Millan et al., 2010; Wernery et al., 2005). It has been reported in saker, peregrines, kestrels, gyrfalcons European sparrow hawks, common buzzards, golden eagles *(Aquila chryseatos)*, red-tailed hawks, red-shouldered hawks, barn owls, Scop's owls, eagle owls, little owls and Ural owls *(Strix uralensis)* from North America, Europe, Asia and Australia (Kris et al., 2013;

Millan et al., 2010; Thomas et al., 2007; Tell et al., 2004; Smit et al., 1987; Sykes, 1982)

Mycobacterium organisms have a long incubation period and regulalry cause disease in adult birds (Johnson, 2009). It has been found that the infection is transmitted through the ingestion of infected prey and close contact between infected individuals (Johnson, 2009). Similarly, Tell et al., (2004) observed that *Mycobacterium avium* infections are more common in gregarious birds since the probability of contracting the disease is higher due to close contact between diseased and healthy birds within a flock. Therefore, the incidence of the disease might be higher in captive raptors, where many birds are housed together in the same enclosure and are forced to interact with one another; in comparison to their free-living counterparts.

Clinical signs are more pronounced in immune-suppressed birds that are subjected to over-crowding in an enclosure, parasitism or suffering from a primary systemic disease (Tell et al., 2004). The affected birds display ill-thrift, loss of appetite, emaciation, non-regenerative anaemia, respiratory distress and death. Pathological findings may include proliferation of granulomas in the intestine, liver, spleen and lungs, hepatomegaly, splenomegaly, coelomic abnormalities and (Johnson, 2009; Tell et al., 2004). The birds have a poor chance of recovering from the disease and euthanasia is usually suggested due to its zoonotic and communicable capacity (Johnson, 2009).

b) Chlamydia psittacii
Chlamydiosis is an important zoonotic disease caused by the intra-cellular bacterium *C. psittacii* (Johnson, 2009; Fowler et al., 1990). It affects a wide range of captive and free-living avian hosts including raptors and has been isolated from peregrine falcons, kestrels, red-tailed hawks, golden eagles, Cooper's hawks, common buzzards, Eurasian sparrowhawks, goshawks, black kites, red kites, tawny owls, barn owls and long-eared owls *(Asio otus)* (Ortega et al., 2012; Schettler et al., 2003; Zacharia & Wernery, 1998; Fowler et al., 1990).

The organism is shed by the infected birds in their mutes or respiratory exudates and transmission occurs either by direct contact with infected birds or ingestion of contaminated meat (Johnson, 2009; Ortega et al., 2012). Not all infected raptors are clinically affected and most birds may serve as asymptomatic carriers of the disease; thereby, fulfilling the role of reservoir hosts as pointed out by Ortega et al, (2012). Birds that are under stress develop clinical symptoms rapidly and may suffer from respiratory distress, diarrhea, weight loss and death (Fowler et al., 1990; Johnson, 2009). Pathological examination may reveal hepatomegaly, splenomegaly and air sacculitis as the most commonly observed signs of the disease (Johnson, 2009). However, not much information in the

literature regarding the prevalence of *Chlamydia* organisms among raptor populations of the world.

c) *Salmonella* spp.

Salmonellosis is caused by several species of gram negative, rod-shaped bacteria belonging to the genus *Salmonella,* with *S. typhimurium* the primary pathogen capable of causing disease in birds. Salmonellosis is a frequently encountered disease among poultry and passerine birds, but not much information is available regarding their prevalence and pathogenicity in Falconiformes and Strigiformes (Thomas et al., 2007). Several serotypes of Salmonella have been isolated from common buzzards, goshawks, Montagu's harriers *(Circus. pygargus)*, red-tailed hawks, Cooper's hawks, Eastern screech owls, tawny owls from Europe and North America (Lamberski et al., 2003; Refsum et al., 2002; Battisti et al., 1998).

Though not frequently reported to cause disease, both clinical and reservoir states of the disease have been observed and the clinically infected birds may show gastrointestinal signs, osteomyelitis, loss of appetite, emaciation and death along with significant neonatal and embryonic mortalities, which can be disastrous in captive breeding facilities (Johnson, 2009; Battisti et al., 1998;). Transmission of the infection is usually through the oral-faecal route and is readily spread by fomites, ingestion of contaminated meat (especially poultry) and contact with rodents (Battisti et al., 1998). Importantly, *Salmonella* spp. infections have significant zoonotic importance but the role of wild raptors in the dispersal of the organism has not yet been well-established and more research needs to be carried out in this regard.

d) *Clostridium* spp.

To date, three main species of *Clostridium* have been found to cause disease in raptors, especially captive birds (Johnson, 2009). *Clostridium* spp are anerobic, gram negative bacteria that can exist in the environment in spore form for extended periods of time and are capable of causing infection when it enters a suitable host (Cooper, 2002). It usually contaminates meat and multiplies during the thawing of frozen meat that is fed to birds at captive centres (Johnson, 2009). Clinical signs of *Clostridium perfringens* may either be acute or peracute. Acute signs include severe gastro-enteritis with reddish-brown diarrhea, regurgitation and weakness, whereas peracute infection results in the death of the bird within hours of disease onset (Cooper, 2002; Johnson, 2009). No specific information regarding the prevalence of clostridium in accipiters is available, except for few cases in hawks, gyrfalcons, saker and peregrines (Cooper, 2002).

Though not an infection of epizootic standards, infection with *Clostridium novyi* or *chauvoei* commonly occur in birds suffering from trauma. Both captive and free-living raptors may suffer from the disease, where puncture wounds get

infected with the organsims resulting in bluish-green discoloration of the skin as a result of necrotising dermatitis (Johnson, 2009). Unless treated, the birds may succumb to the infection due to rapid septicaemia (Johnson, 2009)..

Clostridium botulinum infection is rarely observed in accipiters and no cases have been reported which may be due to the fact that they do not feed on rotting carcasses, which is a primary source of the infection (Johnson, 2009). In addition, it has been suggested that raptors are resistant to *C. tetani* infections, with only a single case in a Saker falcon being reported (Johnson, 2009).

e) Pasteurella multocida

Avian cholera is a highly pathogenic disease of birds caused by the gram negative bacteria, *Pasteurella multocida* (Morishita et al., 1996). Significant mortalities in poultry and waterfowl have been reported from different parts of the world, though the prevalence of the organisms among raptor populations is not well understood (Morishita et al., 1996). Among raptors, *P. multocida* serotype 1 seems to be the most common isolate and affected birds have been found to suffer from either acute or chronic form of the disease, depending on the strain and abundance of the bacteria within their system (Morishita et al., 1997). The disease spreads primarily through the ingestion of contaminated meat from birds suffering from the infection (Morishita et al., 1997). Unlike waterfowl and poultry, Morishita et al (1996) demonstrated that *P. multocida* did not reside as commensal organisms within the digestive tract of raptors and hence the infection had to be acquired either from prey or predators. This is an important finding since outbreaks among raptors have been reported in captive institutes that were fed infected duck meat (Morishita et al., 1997; Morishita et al., 1996). Furthermore, Rosen and Morse (1970) observed that a *Pasteurella* spp. epizootic among montane voles caused the death of 5 Northern harriers following ingestion of the former. Moreoever, poultry and waterfowl are commonly fed to raptors at breeding centres and are commonly preyed upon by free-living raptors as well; this, coupled with the high prevalence of the disease among prey species can increase the risk of a disease outbreak samong raptors (Johnson, 2009; Morishita et al., 1996).

In its acute form, avian cholera can cause death within a few hours following infection due to septicaemia, but in its chronic form, the affected birds show signs of respiratory distress, nasal discharge, conjunctivitis, infra-orbital sinusitis, enteritis, pericarditis, formation of white oesophageal plaques and arthritis (Johnson, 2009; Morishita et al., 1997). Recovered birds survive as carriers for over three months and are capable of spreading the infection to healthy birds and could serve as an epizootic focus, especially in the captive setting (Morishita et al., 1996).

1.5.6 Viruses

a) West Nile Virus

West Nile virus (WNV) infections are caused by viruses belonging to the family *Flaviviridae* and capable of causing disease in humans, horses and over 198 species of birds (Komar, 2003). The virus possesses significant zoonotic potential and wild birds, especially corvids and raptors serve as amplifying hosts for the disease (Fitzgerald et al., 2003). Since being identified in 1937, the most notable epidemic occurred in the US in 2002 that claimed the lives of hundreds of humans, horses and both native and exotic wildlife (Fitzgerald et al., 2003). Strigiformes have been found to be especially susceptible to the virus and has been reported in barn owls, tawny owls, snowy owls *(Bubo scandiacus)*, long eared owls, great horned owls, spotted owls and barred owls to name a few, mainly from the USA (Wunschmann et al., 2005; Komar, 2003). Among Falconiformes, WNV caused significant mortalities among red-tailed hawks, Cooper's hawks, bald eagles, red-shouldered kites, goshawks and golden eagles in 2002-03 in the US (Wunschmann et al., 2004).

The primary mode of transmission occurs through the bite of *Culex* spp mosquitoes that have fed on infected birds or mammals (Reed et al., 2003). The infected birds may be asymptomatic, serving as carriers or suffer acutely, showing signs like lethargy, uncoordinated flight, seizures, tremors, torticollis, head tilt, haemorrhage and death (Fitzgerald et al., 2003). Additionally, an autopsy may reveal hepatomegaly, splenomegaly, myocarditis, encephalitis and wide spread haemorrhage in the coelomic cavity (Fitzgerald et al., 2003).

b) Avian Influenza

Avian influenza is a highly contagious disease responsible for causing mortality in several orders of birds, especially poultry and waterfowl (Johnson, 2009). The disease also has significant zoonotic potential and has claimed the lives of hundreds of humans and captive mammals in Asia following consumption of infected birds (Desvaux et al., 2009; Johnson, 2009).

Avian influenza is caused by Influenza-A virus strains that are categorized as either highly pathogenic (HP) or low pathogenic (LP), based on their pathogenicity and ony the highly pathogenic strains are notifiable (Alexander, 2007). HP avian influenza viruses cause severe multi organ system failures and large-scale motalities whereas, LP avian influenza strains cause mild subclinical infections that manifest as respiratory or reproductive diseases (Alexander, 2007). The Influenza-A virus belonging to the family *Orthomyxoviridae* and is readily transmitted by aerosol contact with respiratory, ocular and gastrointestinal discharges and consumption of meat from infected birds (Johnson, 2009). Migratory wild birds and waterfowl are believed to be primary

carriers of the inection and may have spread the disease over large geographical areas to other avian species like raptors and passerines (Johnson, 2009). Reports of avian influenza in falconiformes are better documented than in strigiformes wherein, only a handful of cases in Bengal eagle owls *(Bubo bengalensis)* and spotted wood owls have been reported from the latter (Redig & Goyal, 2012). Of the Falconiformes, bald eagles, buzzards and crested hawk eagles *(Nisaetus cirrhatus)* in Europe and Asia have been diagnosed with the disease (Redig & Goyal, 2012; Steensels et al., 2007). Recently, avian influenza is also being identified in Houbara bustards *(Chlamydotis undulate macqueenii)*, which are regular prey to falcons in Saudi Arabia and may have even contributed to the death of a saker falcon in 2006 (Bertran et al., 2012; Marjuki et al., 2010)

Rarely, clinical signs such as respiratory distress, conjunctivitis, ocular and nasal discharge, anorexia and neurological signs are observed as sudden death is the usual outcome of infection (Johnson, 2009). Avian influenza has an extremely high morbidity and mortality of 100 % in raptors and its zoonotic potential coupled with the ability to cause an epidemic situation makes this notifiable disease a highly pathogenic (Johnson, 2009).

c) Avipox Viruses

A highly contagious disease of birds, pox infections are caused by avipox viruses belong to the family Poxviridae and have been found to affect at least 60 species of birds extending over 20 families (Johnson, 2009). The disease has been regularly reported in hawks and falcons but is comparatively rare in strigiformes (Johnson, 2009). Cases have been reported from peregrines, sakers, kestrels, lanners and gyrfalcons, barn owls, long-eared owls, great-horned owls, barred owls and Eastern screech owls, goshawks, European sparrowhawks, red-tailed hawks, golden eagles, from North and South America (Vargas et al., 2011; Krone et al., 2004; Ozmen & Dorrestein, 2002; Cooper, 2002; Schoemaker et al.,1998; Deem et al., 1997)

Transmission of the disease is through direct means wherein, the virus may enter a healthy host through abraded skin, inhalation of infected aerosol discharges, ingestion of contaminated meat and also through the bites of arthropods that have previously fed on infected birds (Vargas et al., 2011; Johnson, 2009). The disease manifests itself in two forms- cutaneous and diptheric (Vargas et al., 2011; Johnson, 2009;). The cutaneous form is characterized by epithelial lesions on the non-feathered parts of the body. Though the lesions may not be fatal, invasion by secondary bacterial infections can cause septicaemia and death (Johnson, 2009). The diphtheric form may involve the formation of 'fibrinonecrotic proliferative lesions' in the mucous membranes of the gastrointestinal and respiratory form (Vargas et al., 2011; Johnson, 2009;). The nodules can hamper feeding and respiration depending on

size and severity, leading to emaciation, loss of body condition, dyspnoea, secondary bacterial infections and death (Johnson, 2009). The infections are difficult to treat, highly contagious and require adequate management in captive institutes to prevent the occurrence of disease.

d) Paramyxovirus

Paramyxovirus-1 infections, commonly referred to as Newcastle's disease (NDV), are a common disease of poultry and waterfowl (Jindal et al., 2010; Johnson, 2009). Though vultures are thought to be resistant to the disease, infections have been reported from several species of falconiformes and accipitriformes (Johnson, 2009). Among the strigiformes, NDV has been reported in Eurasian scop's owls, barn owls, tawny owls and great horned owls from Asia, Europe and North America (Jindal et al., 2010; Choi et al., 2008).

Clinical disease begins with gastrointestinal signs like anorexia, vomiting, delayed gastric emptying and paralytic ileus, which then progresses to a neurological form where the birds would exhibit opisthotonus, torticollis, leg paralysis and head-ticking before succumbing to the disease (Wernery et al., 1992). However, in most cases, the only prominent sign of NDV infection is sudden death and diagnosis involves isolation of the virus from vital organs (Chu et al., 1976).

It is not clear how raptors acquire the infection, though it has been stated that consumption of contaminated meat from infected poultry and waterfowl in captive facilities could be the most likely source (Johnson, 2009; Choi et al., 2008). In addition, chickens regularly shed the virus following vaccination and rodents like mice regularly pick up the pathogen, thus when raptors prey on these species, they may contract the disease (Schettler et al., 2003).

1.6.7 Fungal diseases

a) *Aspergillus spp.*
Aspergillus species are ubiquitous fungi, frequently reported in both captive and free-living birds, especially raptors (Garcia et al., 2007; Redig et al., 1980). In fact, Smrco et al (2007) reported that 15-30% of raptor mortalities in captive institutes are from *Aspergillus* spp infections. Unlike other species of birds that are mere carriers of *Aspergillus* spp spores, raptors are especially susceptible to the infection and mortalities have been reported in several falcons, common buzzards, golden eagles, bald eagles, red-tailed hawks, rough-legged hawks, goshawks, black kites, martial eagles and sparowhawks, brown hawk owls and great horned owls (Smrco et al., 2008; Garcia et al., 2007; Redig et al., 1980). Several species of the genus *Aspergillus* have been isolated from birds and mammals so far, of which, *A. fumigatus* is the most commonly encountered pathogen of falcons (Tarello, 2011; Somma et al., 2007). Other species such as *A.*

niger, A. flavus and *A. terreus* have also been reported to cause mycosis in these birds, but possess a low level of incidence (Tarello, 2011).

Aspergillosis is a non-contagious disease and infected birds show signs of lethargy, anorexia, severe respiratory distress and air-sacculitis, although in acute cases, the only presenting sign is rapid and sudden death (Tarello, 2011; Di Somma et al., 2007;). Autopsy may reveal the presence of miliary granulomas in the airways, lungs and airsacs and even fungal growth in respiratory tract and air sacs (Redig et al., 1980).The stress of captivity, restraint and disease exacerbates the risk of contracting aspergillosis and the disease may prevail as either acute or chronic depending on the quantity of spores inhaled (Garcia et al., 2007). Diagnosis of the disease is straightforward, which involves the isolation of spores from affected organs like lungs, airsacs and in chronic cases, other vital organs as well (Tarello, 2011). Suspected birds require intensive antifungal and antibiotic therapy along with isolation to facilitate intensive treatment and management of stress (Redig et al., 1980). The affected birds respond well to anti-fungal treatment, provided they have been diagnosed early and do not suffer from other concomitant infections (Di Somma et al., 2007).

b) *Candida spp.*

Candidiasis is caused by yeast called *Candida albicans*, a commensal organism of birds that colonises in the gastrointestinal tract (Johnson, 2009). The organism is considered to be an opportunistic pathogen unless the host is immune-compromised or stressed, which results in a disease commonly referred to as 'thrush' (Johnson, 2009). The affected birds may demonstrate raised, yellowish plaque like lesions in the oral cavity that resemble those seen in *Trichomonas* or *Capillaria* infections (Johnson, 2009). Though the condition is not lethal, severe lesions can obstruct normal respiration and feeding, leading to respiratory distress, dysphagia, emaciation and even death, if untreated (Johnson, 2009). Diagnosis is made by staining smears of oral swabs or crop washes to demonstrate the organism and by endoscopic examination of the alimentary tract to check for the presence of a diphtheric membrane that possesses a characteristic 'Turkish-towel' appearance (Samour & Naldo, 2002). However, diagnosing the disease may be difficult, since the birds usually suffer from concomitant infections like Capillariasis, Trichomoniasis and *Pseudomonas* infections (Samour & Naldo, 2002). Candidiasis is a frequently encountered disease among raptors, especially captive ones and has been identified in both falconiformes and stigiformes as incidental findings (Johnson, 2009; Garcia et al., 2007; Wieliczko et al., 1997).

1.7 Specific aims of the study

There is very little information in the current literature regarding New Zealand raptor species and causes of morbidity and mortality. Thus, this study had three specific aims: (1) To conduct a retrospective study of post mortem records to

identify the major causes of death in New Zealand's three main raptor species; (2) To analyse archived tissues using molecular methods for the presence of selected pathogens common in New Zealand; and (3) To determine the presence of these pathogens in live populations of captive and wild New Zealand raptors to assess the rate of prevalence in living raptor populations.

1.8 References

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CHAPTER 2: RETROSPECTIVE STUDY OF POST MORTEM FINDINGS IN NEW ZEALAND'S RAPTORS

2.1 Abstract

AIM: To determine the various causes of morbidity and mortality diagnosed in captive and wild New Zealand falcons, Australasian harriers and moreporks received at Massey University, Palmerston North for post- mortem examination between 1990 and 2014.

METHODS: A total of 285 post-mortem reports comprising of 57 (20%) New Zealand falcons, 142 (49.8%) Australasian harriers and 86 (30.2%) moreporks, documented at Massey University's post-mortem database and New Zealand Wildlife Pathology's 'Huia' Database between 1990 and 2014 were reviewed to identify the various causes of mortality in the aforementioned raptors.

RESULTS: Traumatic events appeared to be the primary causes of mortality in New Zealand raptors diagnosed in 47.4% (135/285) of recorded cases. The species most commonly diagnosed with trauma were moreporks [45/98 (52.3%)] and Australasian harriers [74/144 (52.1%)], followed by New Zealand falcons [16/62 (28%)]. Infectious diseases were found to be the second most frequently encountered pathological process in these birds with over 25.3% (72/285) of reported cases. It should be noted that over three quarters of the etiological agents involved could not be determined due to advanced decomposition of the carcass, however, organisms like *Serratospiculum* spp, *Mycobacterium avium, Sarcocystis* spp *and Capillaria* spp were identified through routine parasitological and microbiological techniques. The most reports of clinical infectious disease were noted in New Zealand falcons [21/62 (36.8%)] had followed by moreporks [21/98 (24.4%)] and Australasian harriers [30/144 (21.1%)]. Starvation leading to death was reported in 4.2% (12/285) of cases and there were 32/285 (11.2%) reports of mortality due to other non-infectious causes, including heavy metal toxicity. However, in an additional 18.5% (53/284) of cases, the cause of death could not be determined.

CONCLUSION: This study has identified trauma and infectious agents to be among the primary causes of morbidity and mortality in New Zealand raptors. However, there still remains a considerable lack of information regarding the various infectious diseases affecting these birds. Further research is required to determine the susceptibility of the raptors to various pathogens present in New Zealand.

2.2 Introduction

New Zealand's native raptors face anthropogenic and natural risks that endanger their survival and in some cases, like that of the New Zealand falcon *(Falco novaeseelandia)*, even threaten the species with extinction. The occurrence of anthropogenic causes of death in raptors globally due to electrocution and poisoning have been well documented (Fox & Lock, 1978; Fox & Wynn, 2010). However, information regarding the types of disease affecting New Zealand raptors is scarce. At least 250 raptor post mortem examinations have been documented in the Huia and Massey University post-mortem databases over the past 14 years but only a single clinical case study of serratospiculosis in the New Zealand falcon has been published (Green et al., 2006).

There are three native species of raptors present in New Zealand today, the New Zealand falcon, Australasian harrier (*Circus approximans*), and morepork (*Ninox novaeseelandiae*) and one deliberately introduced species, the little owl *(Athene noctua)*. The New Zealand falcon is New Zealand's only endemic falcon species and it occupies large territories around the eastern North Island and the whole of South Island (Higgins, 1999). Owing to their declining population size as a result of anthropogenic and environmental threats like persecution, introduced predators and habitat degradation, they have been currently classified as 'Nationally Vulnerable/ Nationally Endangered' under the New Zealand threat classification system and as 'Near Threatened' by the IUCN (Butchart & Symes, 2012b; Hitchmough et al., 2007).

The Australasian harrier is New Zealand's largest raptor and can be found inhabiting open farmland, river valleys, tussock grassland and scrubs in both, the North and the South Islands of New Zealand. The availability of abundant prey and carrion and the ability to adapt to variety of landscapes in New Zealand has allowed their populations to thrive, although they do face risks of persecution and death due to the consumption of poisoned carrion (Eason & Spurr, 1995). In recognition of their importance in checking rodent populations and balancing the ecosystem, the harriers have been provided with partial protection under Schedule 2 of New Zealand's threat classification system.

Moreporks are New Zealand's only remaining native owl species that are abundantly found on both the main islands of New Zealand and in parts of Australia and Norfolk Island (Heather & Robertson, 1996). Three sub-species have been identified so far, *N. novaeseelandiae* from New Zealand, *N. n. albaria* from the Lord Howe Island, which has presumably gone extinct and *N. n. undulate* that are found on Norfolk Island (Heather & Robertson, 1996). Moreporks have a healthy population size and do not yet face any major anthropogenic threats that would result in the decline of this species in the near future. However, in the past, it was observed that morepork numbers reduced in Christchurch following the introduction of the little owl in 1930 (Heather &

Robertson, 1996). The little owl was purposely introduced to check the population of introduced passerines and rodents in the early 1900's (Marples, 1942). Although their population failed to establish in the North Island, they soon began to thrive in the South Island; in turn posing a threat to the native morepork (Marples, 1942). Little owls are currently listed under the Least Concern category by the IUCN (Butchart & Symes, 2012).

Infectious and non-infectious diseases have been associated with high rates of mortality in raptors in various parts of the world. For example, infectious diseases reportedly contributed to at least 3% of the total mortalities in Strigiformes and Falconiformes in Florida between 1988 and 1994 (Deem et al., 1998). Wherein, the most frequently occurring ones were of mixed nature involving bacterial, fungal and parasitic organisms such as *Aspergillus* spp, *Trichomonas gallinae* and *Escherichia coli* (Deem et al.,1998). Similarly, mortalities from *T. gallinae* infections were observed in over 40% of the total infectious cases at a wildlife rehabilitation center in Spain between 1995 and 2007 (Molina-Lopez et al., 2011).

Additionally, non-infectious diseases have also significantly contributed to increased mortality of raptors in Asia, North America and Europe, the most significant of which are toxicities. For example, large-scale mortalities were seen in two species of vultures in India as a result of diclofenac toxicity, following the consumption of livestock carcasses containing high levels of the compound (Subramanian, 2011). Similarly, heavy metal and pesticide poisoning has been associated with a gradual population decline of Northern goshawks in Europe while pesticides such as organochlorine compounds have caused deaths in several raptor species in North America (Deem et al., 1998; Mineau et al., 1999; Morrison, 2006).

However, information regarding the effect of non-infectious and non-infectious diseases on New Zealand's raptors is scarce. Thus, the aim of this study is to summarise the findings of post mortem examinations from 1990-2014 of wild and captive New Zealand raptors, including New Zealand falcons, Australasian harriers and moreporks that are available in the New Zealand Wildlife Pathology Database (Huia) and the post mortem database of the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North.

2.2 Methodology

Post mortem reports of New Zealand falcons (n=57), Australasian harriers (n=142) and moreporks (n=86) from 1990-2014 were retrieved from the New Zealand Wildlife Pathology (Huia) Database and the post-mortem database of the

Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North.

2.2.1 Post mortem categories

All reports were compiled and the pathology diagnosed was classified under five categories based on gross lesions, histopathological, microbiological and parasitological analyses as follows:

1. *Unknown cause of death* - this category was assigned for those birds for which the exact cause of death couldn't be ascertained either due to severe autolysis of the carcass, insufficient clinical history or lack of any significant lesions that would indicate trauma or a particular disease process.
2. *Trauma* - this category was assigned for those birds that showed gross physical lesions such as fractures, dislocations and damage to internal organs and musculature that could be attributed to their death, following traumatic events like collisions, gunshot and predation.
3. *Non-infectious disease* - this category was assigned to birds that showed evidence of non-inflammatory or non-infectious disease processes like starvation, gout, arteriosclerosis, toxicities and neoplasms.
4. *Starvation* – This category was assigned to birds that were emaciated as incidicated by poor muscle condition (body condition score of less than 2/5) had no fat reserves and possessed an empty gastrointestinal tract.
5. *Infectious disease* - this category was assigned to birds whose post mortem and histopathological findings indicated pathological processes associated with infectious diseases caused by bacteria, viruses, fungi and parasites. The aetiological agent for all cases was not always identified by adjunctive diagnostic tests.

It is important to note that the findings are reported as frequency of diagnosis. As such, some cases had to be classified under multiple categories, since it was not possible to identify the exact cause of mortality owing to the severity of lesions and advanced nature of a disease process. Cases were identified by accession numbers which enabled those cases reported in both databases to be detected and prevented double counting in our results.

2.2.2 Statistical analysis

Due to low frequencies of some diagnosis categories, Fisher's exact test for independence at a 95% confidence interval and P-value of <0.05 was carried out using the R statistical program (version 3.0.3) to compare the frequency of diagnosis for each category between wild and captive New Zealand falcons, Australasian harriers and moreporks.

2.3 Results

A total of 285 post mortem reports were retrieved that included 57 (20%) New Zealand falcons, 142 (49.8%) Australasian harriers and 86 (30.1%) moreporks, documented over a span of 14 years (1990-2014). The location of death of the birds was mapped to illustrate the spatial distribution of the birds examined. For New Zealand falcons (Figure 1) there was no clear pattern to the geographic locations observed. However, for both Australasian harriers (Figure 2) and moreporks (Figure 3) there was a clear clustering of samples from the Manawatu region of the North Island of New Zealand.
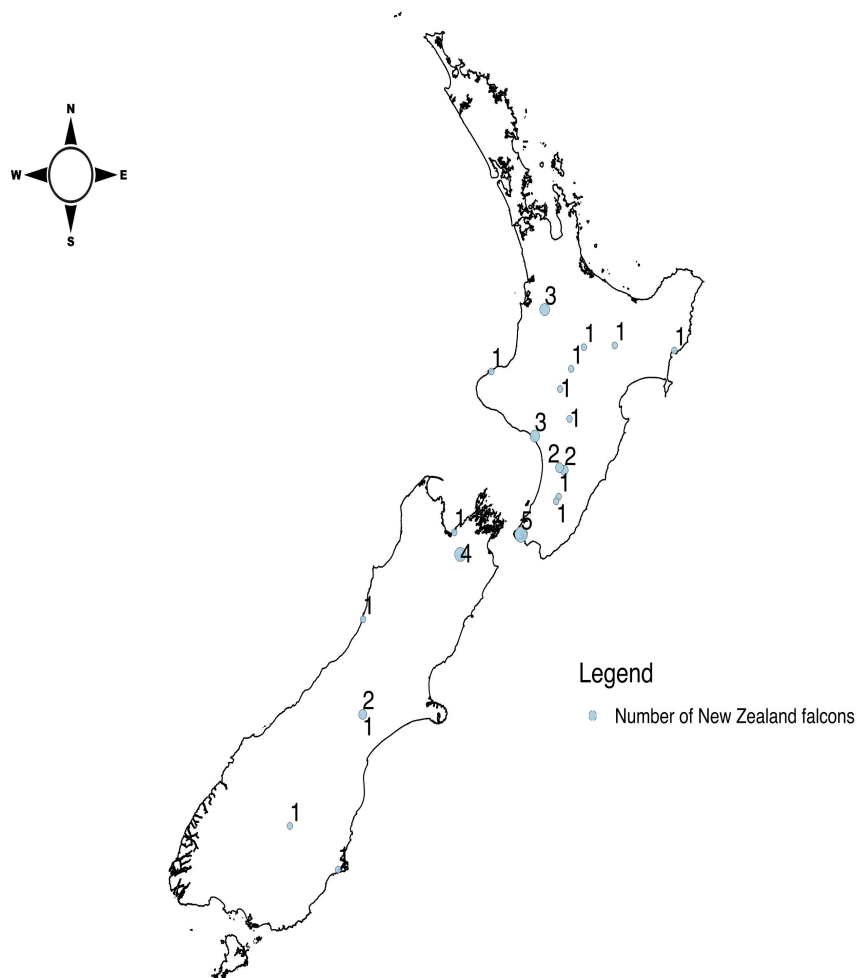
Figure 1: Geographical distribution and number of New Zealand falcon (*Falco novaeseelandiae)* mortalities subject to post-mortem examination in New Zealand between 1990 and 2014.

Figure 2: Geographical distribution and number of Australasian harrier (*Circus approximans)* mortalities subject to post-mortem examination in New Zealand between 1990 and 2014.

Figure 3: Geographical distribution and number of morepork (*Ninox novaeseelandiae)* mortalities subject to post-mortem examination in New Zealand between 1990 and 2014.

2.3.1 Overall frequency of mortality/disease.

The most frequently reported cause of mortality or disease among the three bird species examined was trauma (135/285, 47.4%) followed by infectious agents (72/285, 25.3%) for the timeframe examined (Table 3). In addition, non-infectious diseases, such as toxicity, visceral gout, and neoplasms were observed in 11.2% (32/285) and starvation in 4.2% (12/285) of cases. No diagnosis could be made in 18.6% (53/285) of cases due to advanced decomposition of the carcass or non-remarkable pathology.

Table 3: **Frequency of pathological diagnosis among New Zealand's native raptors from 1990-2014.**

Category of disease	New Zealand falcon (n=57)	Australasian harrier (n=142)	Morepork (n=86)	Total (n=285)
No diagnosis	14 (24.5%)	18 (12.6%)	21 (24.4%)	53
Trauma	16 (28%)	74 (52.1%)	45 (52.3%)	135
Infectious	21 (36.8%)	30 (21.1%)	21 (24.4%)	72
Non-infectious	7 (12.2%)	18 (12.6%)	7 (8.1%)	32
Starvation	4 (7%)	4 (2.8%)	4 (4.6%)	12
			Total	304[#]

[#] Some birds were diagnosed with more than one disease process at post mortem examination, hence the total number of birds is less than the total frequency of disease.

2.3.2 Causes of mortality/disease in New Zealand falcons

Infections (21/57, 36.8%) appeared to be the most frequently reported pathological condition in New Zealand falcons; followed by trauma (16/57, 28%) (Table 3). Among the infectious agents that were identified, the most frequent diseases were serratospiculosis (3/21, 14.2%), aspergillosis (1/21, 4.7%), capillariasis (1/21, 4.7%) and a *Sarcosystis* spp infection (1/21, 4.7%)(Table 4). However, the identity of several microbial and helminth agents was not determined beyond histological diagnosis. Less commonly falcons were diagnosed with diseases of non-infectious nature (7/57, 12.2%); such as arteriosclerosis, visceral gout and neoplasms and a small group were also presumed to have died of starvation (4/57, 7%). There was no significant difference (Fisher's exact p value=0.960; degrees of freedom= 4) in the frequency of the disease categories between wild and captive New Zealand falcons.

2.3.3 Causes of mortality/disease in Australasian harriers

Traumatic events such as predation and vehicular accidents contributed to 52.1% (74/142) of the examined Australasian harrier deaths over the past 14

years, while infectious diseases were responsible for at least 21.1% (30/142) of the total mortalities (Table 3). Among these, mycobacteriosis (3/30, 10%), aspergillosis (3/30, 10%) and capillariasis (4/30, 13.3%) were the most frequently identified infectious diseases, but in over 60% (19/30) of cases of infectious disease, the contributing organisms remained unidentified beyond the histological diagnosis (Table 4).

Of the non-infectious causes of disease among harriers (n= 18), plumbism or lead toxicity was the most common diagnosis in 10/18 (55.5%) cases. Other non-infectious diseases included renal failure and visceral gout (1/18, 5.5%), gall-stones (1/18, 5.5%), renal calcification (1/18, 5.5%) and unidentified neuropathies (3/18, 16.6%). In addition, a small number of mortalities were attributed to starvation (4/142, 2.8%) (Table 3). There was no difference in the frequency of disease category between wild and captive Australasian harriers (Fisher's exact p value=0.132; degrees of freedom= 4).

2.3.4 Causes of mortality/disease in moreporks

Moreporks were most commonly affected by trauma (45/86, 52.3%) of which, collision trauma (vehicles or windows) was found to be the most common form, with at least 16/45 (35%) cases diagnosed with fractures of the skull, cervical vertebrae and shoulder girdle. Unfortunately, although 21/86 (24.4%) of mortalities were considered to be of infectious origin, none of the aetiological agents were identified beyond histological identification as bacterial or mycotic infections or nematodiasis (Table 4). Non-infectious causes of death (7/86, 8.1%) included cholecalciferol poisoning, carcinomas, hepatic lipidosis and cardiomyopathies. A further 4/86 (4.6%) moreporks died of starvation (Table 3). There was no difference in the frequency of category of pathological conditions between wild and captive moreporks (Fisher's exact p value=0.713; degrees of freedom= 4).

Table 4: Frequency of diagnosis of infectious organisms isolated at post mortem examination from New Zealand's native raptors.

Disease	NZ falcon	Australasian harrier	Morepork	Total
Bacterial				
Colibacillosis	1	0	0	1
Mycobacteriosis	0	3	0	3
Unidentified bacterial infections	8	13	12	33
Parasitic				
Capillariasis	1	4	0	5
Sarcocystis	1	0	0	1
Serratospiculosis	3	0	0	3
Trichomoniasis	0	1	0	1
Unidentified helminth infestations	6	6	9	21
Fungal				
Aspergillosis	1	3	0	4
Total	21	30	21	72

2.4 Discussion

Traumatic events were the primary causes of mortality in New Zealand raptors with over 47.4% (135/285) of documented cases that comprised of vehicular accidents, predation, gunshot injuries and collision into windows. The affected raptors suffered from extensive skeletal fractures and rupture of internal organs that either killed them instantly or rendered them unable to fly, which may have led to starvation, disease and death. However, for birds that were presented in the latter condition, it was unclear which event occurred first- trauma followed by disease or vice versa and hence some birds had to be classified into multiple

categories. Moreporks and harriers appeared to be more prone to the risk of trauma than falcons and this may be attributed to their abundant population size and prevalence around urban areas; especially harriers that are exposed to speeding vehicles while scavenging on road kill.

Infectious diseases were found to be the second most frequently encountered pathological condition in these birds with over 25% (72/285) of reported cases. But in at least 75% of cases (54/72) the infectious agents were not identified due to mixed nature of infection, severe autolysis of carcasses or limited adjunctive diagnostic testing. Of note, almost 25% of the recorded moreporks were diagnosed with infectious disease; however, none of the aetiological agents were identified. While this could be lack of sufficient clinical history, an advanced stage of carcass decomposition or mutilation following traumatic events like predation, it is more likely reflective of a bias in the degree of post mortem investigation carried out for the different raptor species. More ancillary diagnostic testing is likely to be used when investigating mortality in a threatened species like the New Zealand falcons compared to the common moreporks and harriers. However, of the infectious agents that could be identified, no reportable diseases exotic to New Zealand were identified and all infectious agents identified have been previously reported in raptors worldwide.

The nematode *Serratospiculum* spp. that localises in the air sacs was isolated from at least three New Zealand falcons in this study. In the affected birds, the parasite caused severe air sacculitis that significantly contributed to their death. This is unlike serratospiculosis cases reported overseas which generally causes no overt signs of disease unless high number of parasites localise within the air sacs (Ward et al., 1971). Green et al., (2011) observed that a naturally infected bird was 'bright and alert' upon admission, however, showed signs of respiratory distress three days later. Following post mortem of the bird, they identified the presence of numerous adult *Serratospiculum* organisms. However, it is not clear whether the falcon in this study showed signs of clinical serratospiculosis following the increase in number of *Serratospiculum* worm-load or due to stress related immune-suppression as it was originally admitted for the treatment of a metacarpal fracture.

In addition to the *Serratospiculum* spp definitively identified, several nematodes were identified in histological sections of the lungs and air sacs of all three raptor species, but their identity was not further determined. Further work is therefore needed to identify the range of respiratory nematodes affecting New Zealand raptors.

In this study, aspergillosis, a disease common among captive raptors, was reported in one New Zealand falcon and three Australasian harriers whose captivity status was not known but may be presumed to be free-living. All

diagnosed cases of aspergillosis were also associated with secondary bacterial infections, traumatic injuries or neoplasms, which is consistent with this mycotic pathogen being primarily an opportunist (Beernaert et al., 2010)

Capillaria spp are common nematodes found inhabiting the gastrointestinal tract of raptors and in most cases this helminth is of low pathogenicity, unless the parasite burden becomes heavy or the host is immune-compromised. The nematode is a common cause of stomatitis in Australasian harriers and is regularly encountered during faecal examination of most live raptors that are admitted to Massey's wildlife hospital ward (personal communication Barbara Adlington, 2014)[2]. Only four Australasian harriers and one New Zealand falcon were severely affected with stomatitis in the records examined for this study. Common causes of stomatitis in raptors include capillariasis, trichominiasis or candidiasis. In the five cases reported here, the diagnosis of stomatitis was confirmed by demonstrating the organism in the buccal mucosa through histopathological techniques. However, the use of histology as the main method of examination may under-diagnose cases of trichomoniasis, as the protozoa are unlikely to survive fixation and processing of tissues, thereby providing false negatives upon analysis.

In addition, there was a single case identifying *Sarcocystis* spp as a suspected incidental finding in a New Zealand falcon. The presence of *Sarcocystis* spp without associated disease have been recognised in a wide variety of New Zealand birds (personal communication, Brett Gartrell, 2015)[3] and in this case the parasite was identified as a single sarcocyst without associated host response in the myocardium of a captive New Zealand falcon that died of unknown causes. In pathogenic *Sarcocystis* infections found in birds overseas, the histology is characterised by pulmonary oedema and presence of merozoites in the lungs (Suedmeyer et al., 2001), therefore it is unlikely that the organism was the cause of death in the New Zealand falcon.

Other factors such as distribution, ecology and population size of the raptor species examined will have greatly influenced the findings of this study. For example, common raptors such as the Australasian harriers and moreporks are more frequently submitted than New Zealand falcons owing to their increased population sizes and abundance in urban areas. Also, behaviours such as scavenging on road kill by harriers are more likely to result in traumatic events and bring the bird in contact with the public, thereby increasing the number of reported casualties. This is reflected in the number of cases documented in the post mortem databases, wherein Australasian harriers were the most frequently

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[2] Personal communication: Ms. Barbara Adlington, Institute of Veterinary Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.
[3] Dr. Brett Gartrell, Institute of Veterinary Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.

occurring, followed by moreporks and New Zealand falcons. In addition, the location of the wildlife hospital at Massey University, Palmerston North may have influenced the number of birds submitted for post mortem, wherein majority of the casualties reported are from the North Island, especially from around the Manawatu-Wanganui region, in comparison to the handful of cases reported from the South Island. Therefore, caution should be used when extrapolating the findings of this study to New Zealand's entire raptor population.

In conclusion, this study revealed that the main causes of mortality in New Zealand's raptor population include trauma and infectious pathogens. However, there is very little information regarding the prevalence of different diseases among our native raptors. Thus additional studies are needed on living raptor populations to understand the prevalence of these infectious and non-infectious diseases and the possible impact these conditions may be having on morbidity and mortality of these birds.

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CHAPTER 3: THE PREVALENCE OF PLASMODIUM SPP. AND TOXOPLASMA GONDII IN ARCHIVED TISSUE SAMPLES FROM NEW ZEALAND RAPTORS.

3.1 Abstract

The two waves of human colonisation of New Zealand included some emerging diseases like avian malaria and toxoplasmosis that were introduced with their exotic bird and mammal hosts. *Plasmodium* spp and *Toxoplasma gondii,* which are causative agents of avian malaria and toxoplasmosis, have a wide host range and have caused mortality in several species of endemic birds. However, no reports of either diseases in New Zealand raptors, namely, the New Zealand falcons *(Falco novaeseelandiae)*, Australasian harriers *(Circus approximans)* and moreporks *(Ninox novaeselandiae)* exist in literature, in spite of the high prevalence of the pathogens among other species of native and introduced wildlife. Therefore, this study was designed to determine the prevalence of these two pathogens in the aforementioned raptors, through a retrospective analysis of archived tissue samples using established molecular techniques. Results of the study revealed that all three species of New Zealand raptors tested positive for the presence of *Plasmodium* spp (10/117; 8.5%) and *T. gondii (*9/117; 7.7%). Strains of *Plasmodium* identified included *P. elongatum* GRW6, *P.* sp AFTRU5 and *P. relictum* SGS1 and GRW4. Moreoever, two Australasian harriers and one morepork tested positive for the presence of both *Plasmodium* spp and *T. gondii* as concomitant infections. However, the pathogenicity of these organisms to the raptors is unclear as none of the post-mortem reports suggested histological evidence of clinical disease associated with *Plasmodium* and *T. gondii* infections. Thus, these results demonstate for the first time, that these two pathogens are present in New Zealand's raptors, however further research is required to determine the prevalence and pathogenicity of these organisms among living populations of these birds in the country.

3.2 Introduction

Emerging infectious diseases are often regarded as a significant threat to wildlife owing to infectious disease outbreaks resulting in large scale mortalities in some wildlife species (Daszak, Cunningham, & Hyatt, 2000), such as the avian malaria outbreak in Hawaii (Marra et al., 2004; Van Riper, Van Riper, Goff, & Laird, 1986), white-nose syndrome in bats in the USA (Blehert et al., 2009) and the global chytrid fungus outbreak in amphibians (Rodder et al., 2009). In New Zealand, two potential pathogens, *Plasmodium* spp and *Toxoplasma gondii,* have

been isolated from several endemic and native birds (Howe et al., 2014; Schoener et al., 2014; Derraik et al., 2008), however, neither pathogen has been reported in New Zealand's raptors, the New Zealand falcons *(Falco novaeseelandiae)*, Australasian harriers *(Circus approximans)* and moreporks *(Ninox novaeselandiae)*. However, both of these pathogens have been reported in related raptor species in other parts of the world (Cabezon et al., 2011; Remple, 2004).

Avian malaria had been identified as an emerging disease in New Zealand, causing significant mortality among native wildlife over the past decade (Derraik et al., 2008). It is an arthropod-borne disease caused by a haemoprotozoan belonging to the genus *Plasmodium* and is mostly transmitted by mosquitoes of the *Culicidae* family (Derraik et al., 2008). In Hawaii, the transmission of avian malaria has led to the extinction of at least 50% of Hawaii's native avifauna, although in conjunction with avian pox (Van Riper et al., 1986). The disease may have established itself in New Zealand with the introduction of *Plasmodium* spp. through introduction of its vectors *Culex quinquefasciatus* from Hawaii and *Aedes australis* from Australia (Derraik et al., 2008).

Of the 38 *Plasmodium* spp. recognised worldwide, 17 lineages have been identified in at least 35 avian species in New Zealand; among which, *Plasmodium elongatum* GRW06 appears to be the most abundant malarial pathogen with an equally extensive host range (Schoener et al., 2014). *Plasmodium* spp. have been isolated from brown and great spotted kiwi *(Apteryx hastii)*, mohua *(Mohua ochrocephala)*, hihi *(Notiomystis cincta)*, blackbirds *(Turdud merula)*, South Island saddlebacks *(Philesternus. c. carunculatus)*, dotterels *(Charadrius obscurus)* and many other species of passerines, though the pathogenicity of the organism may vary from a mild, chronic infection to an acute, highly pathogenic infection depending on the *Plasmodium* spp, the host and its susceptibility to the disease (Schoener et al., 2014).

Toxoplasmosis is a zoonotic disease caused by an Apicomplexan protozoan *T. gondii*, (Dubey, 2008). The organism has a worldwide distribution and is capable of affecting almost all birds and mammals but its pathogenicity varies greatly wherein, some species suffer from clinical infections but many others exist as asymptomatic carriers; the reason for which is still not well understood (Howe et al., 2014; Dubey, 2008). *Toxoplasma gondii* oocysts are shed only by member of the family *Felidae*, which serve as definitive hosts. Ingestion of just one bradyzoite is sufficient for the cat to shed over a million oocysts within 3-10 days post-infection (Dubey, 2008; Miller et al., 1972). These oocysts can remain in the environment for several months until they are ingested by intermediate hosts such as birds; thereby, aiding completion of its life-cycle (Howe et al., 2014).

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Until the arrival of European settlers in the early 19th century, New Zealand was devoid of native felids, thus the presence of *T. gondii* is considered an emerging disease (Bell et al., 1990). A wide host range has allowed *T. gondii* to infect several species of wild birds and mammals in New Zealand. To date, mortalities due to disseminated toxoplasmosis have been reported in kereru *(Hemiphaga novaeseelandiae)*, North Island brown kiwi *(Apteryx mantelli)*, North Island kaka *(Nestor meridionalis)*, captive canaries, and Maui *(Cephalorhynchus hectori maui)* and Hector's dolphins *(C. hectori)* (Howe et al., 2014; Roe et al., 2013; Vickers et al., 1992). However, the prevalence of *T. gondii* among New Zealand's raptors is unknown. Thus, the aim of this study is to determine the prevalence of *Plasmodium* spp and *T. gondii* in New Zealand falcons, Australasian harriers and moreporks through a retrospective analysis of archived tissue samples using established molecular techniques.

3.3 Methodology

3.3.1 Samples

Formalin-fixed or paraffin–embedded tissues of New Zealand falcons (n=35), Australasian harriers (n=46) and moreporks (n=36), which were submitted to Wildbase, Massey University, Palmerston North, New Zealand between 1990 to 2014 were selected for the study (Appendix 1). Samples of liver, lung, heart and spleen tissues were chosen for molecular analysis, in order to determine the presence of *Plasmodium* spp. and *T. gondii*. All tissue samples were fixed in 10% neutral buffered formalin and processed into paraffin blocks for routine histopathological processing. Paraffin-embedded tissues were sectioned at 4μm and stained with haematoxylin and eosin (H&E) for microscopic examination. Immunohistochemical (IHC) examination was performed by the Histology laboratory at Massey University using paraffin-embedded tissues of liver and lung. Tissues were treated and incubated with polyclonal caprine anti-*T. gondii* antibody as described by Roe et al (2013) and examined under a microscope for evidence of staining consistent with disseminated toxoplasmosis.

3.3.2 Molecular analyses

10 μm paraffin embedded slices or <25 mg of formalin fixed liver (n=115), lung (n=108), heart (n=30) and spleen (n=11) samples were used for DNA extraction using a Qiagen DNeasy Kit (Qiagen, Valencia, CA, USA) as per manufacturer's instructions. DNA was stored at -20 °C until needed for polymerase chain reaction (PCR).

A nested PCR protocol was used to determine the presence of the cytochrome b gene of *Plasmodium* spp as described by Hellgren et al (2004) (Appendix 2). The presence of *Toxoplasma gondii* DNA was detected using a nested PCR protocol for the amplification of the *Pppk- dhps* gene as described by Roe et al (2013) (Appendix 3). To determine successful amplification of both PCR protocols, 1.5μl

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of PCR products were run on 1.5% agarose gel (UltraPure Agarose, Invitrogen, Carlsbad, California, USA) containing ethidium bromide (Invitrogen). All *Plasmodium* and *Toxoplasma* spp positive amplicons were purified using PureLink PCR purification kit (Invitrogen,) and subjected to automatic dye-terminator cycle sequencing with BigDye™ Terminator Version 3.1 Ready Reaction Cycle Sequencing kit and the ABI3730 Genetic Analyser (Applied Biosystems Inc, Foster City, California, USA) using the nested forward and reverse primers for confirmation of the genomic sequence. The sequenced products were aligned using the Geneious Pro 4.8.5 (Biomatters Ltd, Auckland, New Zealand) software and submitted to the National Centre of Biotechnology Information (NCBI) blast nucleotide database for confirmation of correct amplification and species identification.

3.3.3 Statistical analysis

Fisher's Exact Test was performed using the R statistical program (version 3.0.3) to test for significant differences (*p<0.05)* in the frequency of *Plasmodium* spp. and *T. gondii* occurrence between raptor species and also between their status of captivity. The captivity status of 10/117 birds could not be determined and were hence omitted from this statistical analysis.

3.4 Results

Tissues from 117 raptors were selected for molecular analysis to determine the presence of *Plasmodium* spp and *T. gondii* (Appendix 1). The total sample set comprised of tissues from three native raptor species; New Zealand falcons (n=35), Australasian harriers (n=46) and moreporks (n=36).

3.4.1 Prevalence and lineages of *Plasmodium* spp.

A total of 10/117 (8.5%) raptors, which comprised of 2/35 (5.7%) New Zealand falcons, 3/46 (6.5%) Australasian harriers and 5/36 (13.8%) moreporks tested positive for the presence of *Plasmodium* spp through molecular analysis (Table 6). Analysis of histopathological sections of the organs that tested positive for *Plasmodium* spp infections by PCR, did not reveal the presence of any exoerythrocytic or intra-endothelial schizonts suggesting that the PCR results were indicative of low-level chronic infections. Of the affected birds, 3/3 (100%) Australasian harriers and 5/5 (100%) Moreporks were free-ranging in nature, whereas 2/2 (100%) New Zealand falcons were reared in captivity (Table 3.1). Following sequencing of the positive amplicons, *Plasmodium* species identified among the New Zealand falcons and Australiasian harriers included *P. relictum* lineage SGS1(99% homology, GenBank AF495571; JX196867) and *P. elongatum* lineage GRW6 (99% homology, GenBank DQ368381)(Table 6). Four moreporks tested positive for the presence of *P. relictum* lineage GRW4 (99% homology,

GenBank AY099041), and one was positive for *P*. sp AFTRU5 (100% homology, GenBank DQ847263).

3.4.2 Prevalence of *Toxoplasma gondii*

Toxoplasma gondii was detected in 9/117 (7.7%) raptors testing positive by PCR and confirmed by sequencing for the presence of *T. gondii pppk-dhps* gene DNA (GenBank U81497) in all positive birds. Australasian harriers were the most commonly infected with 4/46 (8.7%) birds testing positive, followed by moreporks [3/36(8.3%)] and New Zealand falcons [2/35 (5.7%)] (Table 5 and Table 6). *Toxoplasma gondii* was more prevalent in wild New Zealand falcons [2/2 (100%)] Australasian harriers [3/4 (75%)] and moreporks [2/3 (66.6%)] than captive ones [0/2 (0%); 1/4 (25%); 1/2 (33.3%)] respectively (Table 5). Tissues that tested positive for the organism by PCR were also subjected to *T. gondii* specific IHC to demonstrate which of the tissues contained tissue cysts and/or tachyzoites as most paraffin blocks contained samples of multiple tissue types. Of note, there was no evidence of tissue cysts or individual tachyzoites in H&E tissues. Examination of the IHC stained tissues only detected sparse numbers of free tachyzoites in three samples (one harrier, one morepork and one falcon), and no tissue cysts suggesting that the PCR results were indicative of low-level chronic infection with no evidence of disseminated toxoplasmosis.

3.4.3 Co-infections with *Toxoplasma gondii* and *Plasmodium* spp

Among the tested birds, we encountered two free-ranging Australasian harriers and one free-ranging moreporks, which tested positive for the presence of both *T. gondii* and *Plasmodium* spp. Sequencing identified the presence of *P. relictum* (SGS1) and *P. elongatum* (GRW6) in two harriers respectively, while the morepork carried *P. relictum* (GRW4).

Table 5: Summary of the prevalence of *Plasmodium* spp and *Toxoplasma gondii* in New Zealand falcons, Australasian harriers and moreporks in relation to their captivity status.

		Plasmodium spp	*T. gondii*
New Zealand falcon	Wild	0	2
	Captive	2	0
Australasian harrier	Wild	3	3
	Captive	0	1
Morepork	Wild	5	2
	Captive	0	1
Total		**10**	**9**

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Table 6: Summary of molecular analysis on tissue samples from New Zealand raptors to investigate the presence of Plasmodium spp and Toxoplasma gondii.

Species	Total individuals	Number *T. gondii* positive (%) *proportion± 95% CI*	Number *Plasmodium* spp positive (%) *proportion± 95% CI*	*Plasmodium* spp.
New Zealand falcon	35	2 (5.7%) 0.057±0.076	2 (5.7%) 0.057±0.076	*P. relictum* sp SGS1 (n=1); *P. elongatum* sp GRW6 (n=2)
Australasian harrier	46	4 (8.7%) 0.087±0.08	3 (6.5%) 0.065±0.071	*P. relictum* sp SGS1 (n=2); *P. elongatum* sp GRW6 (n=1)
Morepork	36	3 (8.3%) 0.083±0.09	5 (13.8%) 0.13±0.11	*P. relictum* sp GRW4 (n=4) *P. sp.* AFTRU5 (n=1)
Total	**117**	**9 (7.7%)** 0.076±0.048	**10 (8.5%)** 0.085±0.05	

3.4.4 Statistical analysis

There was no significant difference between the prevalence of *Plasmodium* spp and *T. gondii* in the three species of raptor examined in this study (Fisher's Exact Test, *p*=1.0, df= 2).

There was no significant effect of the captivity status of the birds (wild/captive) in relation to the frequency of *Plasmodium* spp. infections among New Zealand falcons (p= 1.0, df=2), Australasian harriers (*p*=1.0, df=2) and moreporks (*p*=1.0, df=2);. Additionally, there was no significant effect of the captivity status of the birds (wild/captive) in relation to the frequency of *T. gondii* infection either amoung New Zealand falcons (*p*=1.0, df=2), Australasian harriers (*p*=0.18, df=2) and Moreporks (*p*=0.27, df=2).

3.5 Discussion

The aim of this study was to determine the prevalence of *Plasmodium* spp and *T. gondii* in New Zealand falcons, Australasian harriers and moreporks through a retrospective analysis of archived tissue samples using established molecular techniques. The results identified four species of *Plasmodium, P. elongatum* (GRW6), *P.* sp AFTRU5 and *P. relictum* (SGS1 and GRW4) and the presence of *T. gondii* within the tissue samples examined. However, it is unclear whether the affected birds suffered from a clinical infection, since their cause of death recorded in the post-mortem databases suggested mortality due to trauma, secondary bacterial infections or plumbism, with no clinical history that may be suggestive of a *Plasmodium* spp or *T. gondii* infection. Moreover, histopathological analysis of *Plasmodium* spp and *T. gondii* positive tissues from this study were negative for both *P.* sp.-associated schizonts and evidence of disseminated toxoplasmosis supporting the clinical history that these two pathogens were not the direct cause of death. The conflicting results between histology and molecular detection is not entirely surprising as both of the nested PCR methods used can detect as little as one parasite's DNA in the sample (Aspinall et al., 2002; Hellogren et al., 2004). In addition, histological techniques, like IHC, can be subjective and dependent on the quality of the tissue, the duration of time preserved in formalin, antibody reactivity, and a reliance on the parasite or lesion being present on the tissue slice presented (Ramos-Vara, 2005).

A total of 17 lineages of *Plasmodium* spp have been identified in 35 species of native and introduced birds in New Zealand so far (Schoener et al., 2014). Of the three lineages were identified in our study, *P. elongatum* lineage GRW6 is perhaps the most abundant of all *Plasmodium* spp in the country, with a wide host range (Schoener et al., 2014; Ewen et al., 2012; Howe et al., 2012). Though, *P. elongatum* infections are usually chronic in nature with a mild level of parasitaemia, pathogenicity may be severe in birds that are immune-compromised due to other infections or subjected to stress following

restraint and transportation; as was observed in South Island saddlebacks *(Philesternus carunculatus)* that succumbed to avipox and *P. elongatum* co-infections following transportation (Alley et al., 2010;). This haemoprotozoan has also been associated with the death of a brown kiwi *(Apteryx mantelli)* in the past (Banda et al., 2013).

Similarly, *Plasmodium* sp AFTRU5 appears to also be cosmopolitan in nature and has been identified in the blood of a wide range of New Zealand bird species (Schoener et al., 2014). This *Plasmodium* species is closely related to *P.* sp LINN1, cause has caused mortality in both a European blackbird and a great spotted kiwi (Howe et al., 2012; Alley et al., 2012). However, the distribution, pathogenicity and prevelance of *P.* sp AFTRU5 in New Zealand birds remains unclear.

Plasmodium relictum lineages SGS1 and GRW4 are cosmopolitan in distribution; however, they differ in their geographical distribution (Palinauskas et al., 2008). Lineage SGS1 is prevalent in tropical Africa, Europe, East Asia, South Korea and the Polar Regions, while GRW4 is found more in tropical Africa and oceanic islands like Hawaii (Palinauskas et al., 2008). *Plasmodium relictum* lineage SGS1 has been observed in at least 29 passerine species in Europe and Africa but its pathogenicity is debatable, since experimental infections with the parasite failed to cause mortality in birds like siskins *(Carduelis spinus)* and crossbills *(Loxia* spp*)*, although the birds suffered from high parasitaemia, decreased haematocrit values and hypertrophy of internal organs (Palinauskas et al., 2008). Moreover, passerines like starlings and sparrows were found to be completely resistant to clinical *P. relictum* SGS1 infection. However *P. relictum* lineage GRW4 is a pathogenic strain and is known to have caused mortalities of up to 90% in native Hawaiian birds in the 19th century, following its introduction (Van Riper et al., 1986).

Although several species of *Plasmodium* have been found to infect raptors there is little information regarding the prevalence and pathogenicity of specific strains of *Plasmodium* spp (Perez-Rodrigues et al, 2013; Salakij et al., 2012; Ortego et al., 2007). However, Ortego et al. (2007) and Krone et al. (2001) suggest that the prevalence of *Plasmodium* spp is lower in birds of prey compared to other avian species, possibly due to decreased susceptibility to disease and geographical distribution. Further research is required to determine the role played by New Zealand's raptors in the transmission of *Plasmodium* spp and its effect on New Zealand's raptors; especially moreporks, since all the affected birds were found carrying the potentially pathogenic GRW4 lineage that was also asociated with the decline of Hawaii's avifauna.

Toxoplasma gondii has been documented in several species of wild birds and animals in New Zealand, with varying degrees of pathogenicity noted within different host species (Howe et al., 2014; Roe et al., 2013). In spite of its frequent occurrence, the pathogen has not been previously reported in New Zealand's raptors. Australasian harriers recorded a higher frequency of *T. gondii* infection compared to New Zealand falcons and moreporks. Since oral ingestion of infective stages is the only mode of disease

transmission and the harriers' diet extends to scavenging of carcasses, it is therefore possible that these birds have a high risk of exposure when scavenging on infected carcasses of rabbits and possums. Unfortunately, due to time constraints, genotyping of the isolates was not able to be performed. Future genotyping may help to attribute the source of *T. gondii* infection.

Globally, raptor populations possess high morbidity and low mortality to *T. gondii* infections (Aubert et al., 2008; Lindsay & Blagburn, 1999). They are generally resistant to clinical manifestations of the disease, except for a handful of cases, where a bald eagle *(Haliaeetus leucocphalus)* and a barred owl *(Strix varia)* succumbed to the infection (Szabo et al., 2004; Mikaelian et al., 1997). Raptors are most likely to acquire the infection by consuming bradyzoites encysted in infected tissues and this could be a possible mode of transmission among captive birds, if fed infected game birds and mammals (Aubert et al., 2008). Therefore, an assessment of the prevalence and genotype of *Toxoplasma gondii* in New Zealand's raptor populations would provide insight into the prevalence of the pathogen among its prey species as well.

An interesting observation made during the course of our study was the presence of concomitant infections with both *P. elongatum* and *T. gondii* organisms in two harriers and a morepork each, although, histopathological analysis suggested that none of these birds suffered clinically from either disease. Mixed infections in raptors have been reported in accipitriformes and strigiformes in the past, but between *Plasmodium, Haemoproteus, Leucocytozoon* and *Trypanosoma* species (Munoz et al, 1999). Information regarding co-infections between *Plasmodium* and *T. gondii* in raptors is unavailable and it is unknown whether the presence of these two organisms can contribute to clinical disease or mortality, as observed in the case of concurrent avipox and *Plasmodium* co-infections in South Island saddlebacks (Alley et al., 2010).

To our knowledge, this is the first study identifying the presence of *Plasmodium* spp and *T. gondii* among New Zealand's raptors. However, the pathogenicity of these organisms to the raptors is unclear as acute infection with *Plasmodium* nor *T. gondii* were recorded in the post mortem records or histologically observed in this study. It could be possible that the disease may have rendered the birds weak or immune-compromised, which made them susceptible to trauma and secondary bacterial infections. Therefore, both pathogens should be considered as part of the diagnosis and treatment plan for sick raptors. In addition, further research is also required, to understand the prevalence and impact of both diseases in the country's wild raptor populations.

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CHAPTER 4: A SURVEY OF WILD AND CAPTIVE NEW ZEALAND RAPTORS FOR SELECTED PATHOGENS, INCLUDING *PLASMODIUM* SPP, *TOXOPLASMA GONDII*, *SALMONELLA* SPP, HELMINTH AND PROTOZOAL PARASITES.

4.1 Abstract

There is very little information regarding infectious diseases affecting New Zealand's raptors, the New Zealand falcon *(Falco novaeseelandiae)*, Australasian harrier *(Circus approximans)* and morepork *(Ninox novaeseelandiae)*. However, raptors around the world are susceptible to a range of infectious diseases, including diseases such as avian malaria and toxoplasmosis, which have been encountered in various other species of New Zealand's avifauna. In a prior study using molecular analysis of archived post mortem tissues I detected the presence of both *Plasmodium* spp and *Toxoplasma gondii* in all three species of raptors. Thus, the aim of this study was to determine the prevalence of several important pathogens in 29 live raptors, that were either admitted to Wildbase Hospital (Massey University, Palmerston North) or housed at Wingspan- Birds of Prey Research Center. None of the 29 raptors sampled were positive for the presence of *Plasmodium* spp DNA. However, one New Zealand falcon, one Australasian harrier and one morepork tested positive for the presence of *T. gondii* DNA. There was no statistically significant effect of the status of captivity on infection with *T. gondii* for New Zealand falcons (*p*= 0.45), Australasian harriers (*p*=1.0) and Moreporks (*p*=1.0). Faecal flotation and microscopic examination of wet smears of the faeces revealed the presence of significant numbers of *Capillaria* spp eggs in seven birds (four harriers, one falcon, and two moreporks). However, no other helminth or protozoal pathogens such as *Caryospora* spp, *Serratospiculum* spp, and *Trichomonas* spp were identified. Microbiological culture of the faeces of all birds was negative for *Salmonella* spp. No evidence of clinical disease was associated with the *Toxoplasma gondii* or *Capillaria* spp detected.

4.2 Introduction

Globally, a wide range of infectious agents, including protozoa, nematodes, bacteria, viruses and fungi, have been recorded as pathogens and parasites of

Chapter 4: Disease survey of wild and captive raptors

raptor species. However, very little information regarding the diseases affecting New Zealand raptors is available in literature. The only disease reported so far is an incidental finding of serratospiculosis in an adult male falcon suffering from a traumatic fracture of the left wing (Green et al., 2006). A radiographic examination revealed the presence of opaque masses in the caudal abdominal air sacs and progressively worsening respiratory signs led to its death shortly after admission. Necropsy revealed the presence of several nematodes belonging to the species *Serratospiculum guttatum* (Green et al., 2006). Previously, I undertook a retrospective analysis of archived post-mortem tissues at Massey University, Palmerston North which identified *Toxoplasma gondii, Plasmodium elongatum* GRW6, *P.* sp AFTRU5 and *P. relictum* SGSI and GRW4 in New Zealand falcon *(Falco novaeseelandiae)*, Australasian harrier *(Circus approximans)* and morepork *(Ninox novaeseelandiae)* (Chapter 3).

Thus, the aim of this study was to determine the prevalence of several important pathogens in live raptors, including *Plasmodium* spp, *T. gondii, Caryospora* spp, *Salmonella* spp *and Trichomonas* spp using established molecular, microbiological and parasitological techniques.

4.3 Methodology

4.3.1 Samples

A total of 29 raptors were sampled opportunistically over 2014, including New Zealand falcons (n=15), Australasian harriers (n=8), and moreporks (n=6). Our sample set included raptors from three locations in New Zealand; Wingspan - Birds of Prey Research Center, Rotorua (n=17), birds admitted to Wildbase Wildlife Hospital, Massey University, Palmerston North (n=11) and one wild falcon residing in the Kaingaroa forest region, Bay of Plenty. The raptors sampled at Wildbase included birds from both wild and captive origin, while those from Wingspan were held and mostly reared in captivity.

Blood samples (n=24) were collected by manually restraining a bird and drawing 0.1-0.2ml of blood either from the medial metatarsal vein or the brachial vein using 2ml syringes fitted with a 25 gauge needle. Blood collected was immediately transferred to heparin tubes (BD Vacutainer® Lithium Heparin™, USA) and frozen at -80°C until molecular analyses could be performed.

Faecal samples (n=29) were collected by two methods:

1) scooping fresh faecal material from cages/aviaries into non-sterile plastic containers, for parasitological analysis. All faecal samples were frozen at -20°C until ready for analyses.

2) swabbing the cloaca of birds following restraint, using sterile swabs and immediately storing them in transport media (CultureSwab Plus Amies Gel with Charcoal, Becton Dickinson, New Jersey, USA) for transport to a commercial veterinary diagnostic laboratory (New Zealand Veterinary Pathology) for microbiological culturing of *Salmonella* spp.

Throat swabs (n=14) were obtained by swabbing the pharyngeal region of the oral cavity using sterile swabs and immediately using them to inoculate *Tritrichomonas foetus* test pouches (InPouch TF, Biomed Diagnostics, Oregon, USA). The pouches were stored at 37°C up to two weeks after collection to facilitate growth of trichomonads and were checked daily for their presence under a light microscope (x10 magnification) (Bunbury et al., 2005). Microscopic examination of fresh smears for the presence of *Trichomonas* spp. was not undertaken, since visualization of the smear must be done within ten or tweny minutes following collection to prevent the risk of false negatives, as the organism loses its viability over time (Schwebke & Burgess, 2004). Moreoever, this test possesses a low sensitivity of 60-70 %, furthur increasing the probability of fale negatives (Schwebke & Burgess, 2004).

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4.3.2 Molecular analyses

Extraction of DNA was performed on the 24 blood samples using a Qiagen DNeasy Kit (Qiagen, Valencia, CA, USA), as per manufacturer's instructions for nucleated whole blood. DNA was stored at -20°C until needed for polymerase chain reaction (PCR). As described in Chapter 3, a nested PCR for determination of the *cytochrome b* gene of *Plasmodium* spp and *Pppk-dhps* gene of *T. gondii* was carried out using protocols adopted by Hellgren et al (2004) and Roe et al (2013), respectively. Procedures and tools used for the purification of positive amplicons and subsequent sequencing for confirmation of amplification and species identification were identical to those described in Chapter 3.2.2 of this thesis.

4.3.3 Parasitological analysis of faeces

Faecal flotation technique was adopted to demonstrate helminth and protozoal parasites, using a procedure described by Faust et al (1939) with minor modifications. A mixture of 0.5g faeces and 33% zinc sulphate solution was prepared in a bowl and strained into test tubes until a meniscus formed at the top. The use of a strainer was necessary to remove debris in order to aid better visualisation of the eggs. Then, a clear 20x20 mm coverslip was placed on top of the tube and centrifuged at 1,200 rpm for 5 minutes. After centrifugation, the coverslip was carefully lifted off the tube and placed on a clear glass slide to be visualised under a microscope.

4.3.4 Microbiological culturing of *Salmonella* spp

Cloacal swabs of raptors were stirred in 9ml vials of Selenite Cystine and Tetrathionate broth (Fort Richards, Auckland, New Zealand), incubated at 35°C for 24 hours, plated onto Xylose lysine Deoxycholate (XLD) agar (Fort Richards) and incubated at 35°C for 24 hours (Moriarty et al., 2011). Suspected colonies were re-streaked onto XLD to obtain a pure culture.

4.3.5 Animal ethics

Massey University's Animal Ethics Committee (MUAEC, 2014, Protocol No. 14/71) and the Department of Conservation, under its 'Wildlife Act Authority for wildlife not located on public conservation land' (Authorisation No. 39540-RES) approved the sampling of 50 captive birds (New Zealand falcons, Australasian harriers and moreporks).

4.4 Results

Biological samples such as blood (n=24), faeces (n=29) and throat swabs (n=14) from 29 individual raptors were analysed to investigate the presence of *Plasmodium* spp, *T. gondii*, *Salmonella* spp, *Caryospora* spp and *Trichomonas gallinae*. The birds sampled at Wingspan-Birds of Prey Research Center, Rotorua (n=17) appeared healthy with no overt signs of clinical illness. However, the raptors sampled at Wildbase Hospital, Massey University, Palmerston North (n=11), were admitted with signs of trauma, starvation or other undetermined illnesses.

4.4.1 Prevalence of *Plasmodium* spp

No *Plasmodium* spp DNA was detected in any of the three sampled species using molecular analysis of blood samples from New Zealand falcons (n=11), Australasian harriers (n=8) and moreporks (n= 5).

4.4.2 Prevalence of *T. gondii*

Blood samples from one New Zealand falcon [1/11 (9.0%)], one Australasian harrier [1/8 (12.5%)] and one morepork [1/5 (20%)], tested positive for the presence of *T. gondii* DNA by PCR. Subsequent sequencing of positive amplicons confirmed the presence of *T. gondii pppk-dhps* gene DNA (GenBank U81497) (Table 7). Of the positive birds, the harrier was free-ranging from Feilding, Manawatu-Wanganui region and was admitted to Wildbase wildlife hospital following trauma. The New Zealand falcon and the morepork were captive–reared birds housed at Wingspan with no clinical signs of ill-health.

4.4.3 Parasitological analysis

No coccidial oocysts were observed in any of the faecal samples (n=29), following faecal flotation. However, helminths belonging to the genus *Capillaria*

spp [8/29 (27.5%)] were present as incidental parasites (Table 7). Among the wild birds, *Capillaria* spp were seen in two harriers [2/12 (16.6%)] under hospital care at Massey University, and among captive birds, *Capillaria* was seen in faecal samples of 2/17 (11.7%) Australasian harriers, 1/17 (5.8%) New Zealand falcon and 2/17 (11.7%) moreporks from Wingspan.

4.4.4 Microbiological analysis

No *Salmonella* spp was cultured from the 29 cloacal swabs and the only observable microbial growth present was of suspected *Escherichia coli* or other bacterial or fungal colonies on 6/29 (20.68%) plates, which were not identified further.

No *T. gallinae* organisms were detected in the Tritrichomonas pouches after two weeks of culture.

Table 7: Results of laboratory analysis conducted on blood and faecal samples from live New Zealand raptors.

Species	New Zealand falcon	Australasian harrier	Morepork
Individuals (n)	15	8	6
***Toxoplasma gondii* n(%)**	1 (9%)	1 (12.5%)	1 (20%)
Prevalence ± 95% CI	0.09±0.16	0.12±0.22	0.20±0.35
***Capillaria* spp**			
n(%)	1 (6.6%)	4 (50%)	2 (33.3%)
Range (oocysts/gram)	0-65	20 - 430	20- 1630
Prevalence ± 95% CI	0.06±0.12	0.5±0.49	0.33±0.37

4.5 Discussion

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The aim of this study was to survey live raptors in New Zealand for the prevalence of selected pathogens including *Plasmodium* spp, *T. gondii*, *Caryospora* spp, *Salmonella* spp and *Trichomonas* spp. Of these pathogens, we only identified the presence of *T. gondii* in a single wild Australasian harrier and from a captive New Zealand falcon and a morepork.

Plasmodium spp are well established in New Zealand (Schoener et al, 2014) and the fact it was not detected in this small sample set is somewhat surprising given that we have previously identified a prevalence of 7.7% in archived post mortem samples (Chapter 3). In interpreting this result, it is also important to note that 17 birds were housed in captivity in aviaries that were designed to prevent the entry of vectors, such as mosquitos, which probably reducing the exposure of these birds to the pathogen. Given the low sample size of this study, care should be taken before drawing any major conclusions. However, if using my previous prevalence estimate from the archived post mortem tissues, I expected a 95% chance of at least two of the birds sampled to be positive for *Plasmodium* spp DNA given our sample size. The negative results of this survey may have been due to sampling chance, or variations in prevalence between live and dead birds, or between seasons, geographic region and vector abundance (Schoener et al., 2014; Ewen et al., 2012; Tompkins & Gleeson, 2006).

At least 17 lineages of *Plasmodium* spp have been identified in over 35 species of birds in New Zealand of which, 3 lineages from two species of *Plasmodium- P. elongatum* lineage SGS1, GRW6 and *P. relictum* lineage GRW4 were identified in New Zealand falcons, Australasian harriers and moreporks, through a retrospective analysis of post mortem tissues (Chapter 3). Various *Plasmodium* spp have also been identified in several species of falcons and accipiters in Europe and North America, but not much is known about the different lineages affecting strigiformes (Perez-Rodriguez et al., 2013; Ortego et al., 2007). Therefore, more extensive sampling of wild raptors from different geographical regions of New Zealand would provide more accurate information regarding the prevalence of *Plasmodium* spp. among New Zealand raptors.

Toxoplasma gondii is a ubiquitous Apicomplexan protozoan, with a wide host range. In New Zealand, fatal toxoplasmosis has been reported in kereru *(Hemiphaga novaeseelandiae)*, a North Island brown kiwi *(Apteryx mantelli)*, and a North Island kaka *(Nestor meridionalis)* (Howe, et al., 2014). A previous retrospective analysis of archived post mortem tissues at Massey University, Palmerston North, New Zealand confirmed a prevalence of 7.7% in New Zealand falcons, Australasian harriers and morepork, although there was no associated histological evidence of disease in the archived tissue samples. In this survey, I identified the organism in all three species of raptors at a low prevalence, but again, the pathogenicity of the organism in these raptors is unclear since none of the affected birds displayed clinical signs of illness. Clinical toxoplasmosis in

birds has been associated neurological deficits, blindness, conjunctivitis and gastrointestinal signs like diarrhoea, which have been documented in other avian species (Rigoulet et al., 2014; Vickers et al., 1992). However, raptors are generally believed to be resistant to clinical toxoplasmosis, since only two case studies involving mortality in raptors from toxoplasmosis have been recorded, involving a bald eagle *Haliaeetus leucocephalus* and a barred owl *Strix varia* (Szabo et al., 2004; Mikaelian et al., 1997). Moreover, experimental infections with the pathogen have also failed to elicit clinical symptoms despite seroconversion occurring in several species of owls (*Bubo virginianus, Strix varia, Asio otus*), American kestrel (*Falco sparverus*) and red-tailed hawks (*Buteo jamaicensis)* after being fed tissue containing *T. gondii* cysts (reviewed in Dubey, 2002).

Since one mode of transmission of the protozoan is through ingestion of bradyzoites or tissue cysts from contaminated meat, the prevalence of *T. gondii* DNA in raptors might be correlated with the prevalence of the pathogen in their prey species as well. The captive New Zealand falcon and morepork that were positive for the organism in our study may have picked up the organism through the ingestion of contaminated meat, since these birds are occasionally fed game animals such as pigeons, mallard ducks, canadian geese and small passerines donated by local hunters (personal communication, I. Smets, 2014)[4].

Salmonellosis is a rare disease of raptors, observed more frequently among free-living birds than captive ones and has been reported from raptors all around the world although with low levels of pathogenicity (Molina-Lopez et al., 2011; Reche et al., 2003). The disease is of zoonotic importance and a pathogenic strain of *Salmonella* spp. caused significant mortalities in wild birds such as sparrows and humans in New Zealand in 2002 (Alley et al., 2002). In this study, we did not culture any *Salmonella*-like organisms from cloacal swabs.   This may be due to the fact that these birds were fed meat that was frozen for extended periods of time prior to being thawed for feeding and this may have hampered the survival of the bacteria in meat (personal communication, Andrew Thomas, 2014)[5]. The prevalence and pathogenicity of *Salmonella* spp in New Zealand falcons, Australasian harriers and moreporks is still unknown, since post mortem records at Massey University and the Huia post mortem database do not contain any reports citing the incidence of mortality in these birds due to *Salmonella* spp.

Trichomoniasis caused by *T. gallinae* is a pathogenic disease of raptors capable of causing mortality. The disease manifests as caseous oral plaques in the buccal cavity which hampers swallowing, ultimately resulting in death of the affected birds (Atkinson et al., 2008; Samour & Naldo, 2003; Cooper & Petty, 1988). We adopted the suggested diagnostic method of culturing pharyngeal swabs of birds

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in test pouches and checking for motility of the parasites under a microscope (Bunbury et al., 2005). But the organism could not be demonstrated among any of the birds tested. The disease is of communicable nature and we were expecting to observe its presence among the captive birds that were sampled. The lack of detection of *T. gallinae* among these raptors may be truly negative or be false negatives due to the fragile nature of the organism and that the culture pouches are made for bovine strains of *Tritrichomonas* spp.

Coccidiosis due to *Caryospora* spp. is a commonly encountered disease of captive raptors in Europe, Middle East and North America (Volf et al., 2000) but it has yet to be identified in New Zealand. In this study, no identifiable coccidial oocysts were observed following analysis through faecal flotation. However, oocysts resembling *Capillaria* spp. were observed in 24% of the sampled birds. In spite of the absence of the *Caryospora* spp. in the birds sampled in our study, given the small sample size of our study, care should be taken before extrapolating these results to wider populations of New Zealand raptors.

In summary, this small survey detected *Toxoplasma gondii* and *Capillaria* spp infection in all three species of New Zealand's raptors. There was no evidence of *Plasmodium* spp, *Salmonella* spp, *Caryospora* spp and *Trichomonas* spp infection among the sampled birds but these results are probably limited by the small sample size and predominance of captive birds sampled. Thus further studies involving a greater sample size of wild birds is required to provide accurate information regarding the prevalence of these pathogens among New Zealand's wild populations of raptors.

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CHAPTER 5: GENERAL DISCUSSION

5.1 Overview of major findings

Infections were established as the second most common causes of death among New Zealand raptors through a retrospective analysis of post-mortem records to identify various causes of mortality in New Zealand's raptors. Additionally, *Plasmodium* spp and *Toxoplasma gondii* infections that been observed as emerging pathogens in several native and introduced birds in the country, have never been reported among New Zealand's raptors (Howe, et al., 2014; Schoener, et al., 2014). However, both pathogens were identified in all three raptor species-the New Zealand falcon *(Falco novaeseelandiae),* Australasian harrier *(Circus approximans),* and morepork *(Ninox novaeseelandiae)* through molecular analysis of archived post mortem tissues, though only *T. gondii* was seen in a small number of live captive and wild raptors. Importantly, infection was not associated with evidence of concurrent disease in neither the post mortem samples nor the live bird survey. The presence of these two pathogens among raptors opens new areas of research, which may be required to determine their pathogenicity, their effect on survivability of re-introduced birds and sub-clinical effects of these diseases on general fitness and reproduction among New Zealand's raptors, to name a few.

5.1.1 Identification of major causes of mortality in New Zealand's raptors

A retrospective study of post-mortem reports for New Zealand's raptors between 1990 and 2014 (Chapter 2) revealed that traumatic events were the most common diagnoses in these birds. It was observed that events like vehicular accidents, predation, collision into windows and gunshot injuries were the most frequently encountered forms of trauma. These events point towards the anthropogenic threats faced by New Zealand raptors in the form of habitat alteration and predation.

Infections were the second most common diagnoses, with diseases such as serratospiculosis, mycobacteriosis, capillariasis and trichomoniasis being documented in raptors (but not necessarily in all three species). Significantly, diseases like avian malaria and toxoplasmosis have been encountered in various native and introduced New Zealand birds, with significant rates of mortality (Howe et al., 2014; Schoener et al., 2014), but neither were identified and reported in the post mortem reports examined.

Other diagnoses reported in the raptors included non-infectious diseases like cholecalciferol poisoning, carcinomas, non- infectious causes of organ failure and atherosclerosis. There were many reports wherein the exact cause of death could not be ascertained owing to the lack of sufficient clinical history and advanced state of decomposition of the carcass during submission, or limited post mortem investigation.

5.1.2 Determination of the presence of *Plasmodium* spp and *Toxoplasma gondii* in archived New Zealand raptor tissues

Infectious diseases are among the major causes of mortality in New Zealand raptors (Chapter 2). There is an increasing prevalence of diseases like avian malaria and toxoplasmosis among New Zealand's avifauna (Howe et al., 2014; Roe et al., 2013; Derraik et al., 2008; Vickers et al., 1992) that was surprisingly not reflected in the retrospective review of the New Zealand raptor reports. The raptors' distribution and diet means that exposure to both pathogens is likely; avian malaria via bites from infected mosquitos and Toxoplasma through ingested prey. This raised the question as to whether New Zealand's raptors were being infected asymptomatically or were resistant to infection.

I therefore investigated for the presence of these pathogens in archived post-mortem tissues of New Zealand raptors stored at Massey University, Palmerston North (Chapter 3). I revealed the presence of both *Plasmodium* spp and *T. gondii* in all three raptor species. Different strains of both *P. elongatum and P. relictum* were identified among the birds of which, all moreporks tested positive for the more infectious *P. relictum* GRW4. This strain has been associated with heavy avian mortalities in Hawaii wherein, over 90% of the native passerine population succumbed to the disease (Marzal et al, 2015). The other strains identified included *P. elongatum* GRW6 and *P. relictum* SGS1. Interestingly, one New Zealand falcon appeared to carry both the aforementioned strains, but histological examination of the infected tissue samples did not show any evidence of pathology associated with avian malarial disease. The most likely explanations for this finding is that the birds were in a chronic stage of asymptomatic infection with low numbers of organisms at the time of their death from other causes. However, it may be possible that avian malaria infection has resulted in lowered fitness and a greater likelihood of mortality from other causes and this possibility is worthy of further investigation.

I also identified *T. gondii* in the archived tissues of all three species of raptors tested. Australasian harriers recorded a higher frequency of infection compared to moreporks and falcons. This may be attributed either to their larger sample size or feeding behavior, wherein, scavenging on dead prey can increase the risk of exposure by bringing them in contact with carcasses of infected animals like

possums, hares and rabbits, since the only mode of transmission is through ingestion of bradyzoites from infected tissues or ingestion of oocysts from contaminated soil (Dubey, 2008). Histological and immuno-histochemical examination of the infected tissue samples showed no evidence of pathology associated with clinical toxoplasmosis either. Raptors are generally considered resistant to clinical toxoplasmosis, except for two reported cases of mortality in a Bald eagle *(Haliaeetus leucocphalus)* and a Barred owl *(Strix varia).* Therefore, while the susceptibility of New Zealand's raptors to toxoplasmosis and the pathogenicity of the organism towards them is not yet fully determined (Mikaelian, et al., 1997; Szabo, et al., 2004), I found no evidence that *Toxoplasma gondii* is a major concern to New Zealand raptor populations. The most likely explanation for my results is that the raptors infected with *Toxoplasma gondii* had asymptomatic infections. Two Australasian harriers and a morepork were also found carrying mixed infections of *Plasmodium* spp and *T. gondii*, but there is no evidence they suffered clinically from either. It is likely that NZ raptors are an incidental or dead-end host for *Toxoplasma gondii.*

5.1.3 Determination of the presence of *Plasmodium* spp and *T. gondii* in live New Zealand raptors

Once the presence of *Plasmodium* spp and *T. gondii* was established in New Zealand raptor populations, I surveyed for these pathogens among live birds, both captive and wild. Blood samples collected, were subjected to molecular analysis through PCR and it was found that none of the sampled birds carried *Plasmodium* spp, but one wild Australasian harrier and one captive New Zealand falcon and one morepork were each infected with *T. gondii.* The source of infection for the wild harrier could be the contaminated carcasses which it regularly scavenges upon and the captive birds may have contracted the infection through consumption of contaminated game birds and mammals, which are a regular feature of their diet (personal communication, Ineke Smets, 2014). However, none of the positive birds showed signs of illness that may be attributed to *T. gondii* infections (Mikaelian et al., 1997; Rigoulet et al., 2014).

Since pathogens like *Serratospiculum, Caryospora* spp, *Trichomonas gallinae* and *Salmonella* spp have been reported in global raptor populations and among New Zealand's avifauna, I also investigated their presence among the raptors in concern as well. However, none of the sampled birds tested positive for these organisms upon microbiological and parasitological analysis. I did detect a high prevalence of capillariasis in all three species examined, especially in the captive birds. Though capillariasis manifests as a sub-clinical infection in raptors, it can be exacerbated in the presence of a primary disease or stress (Redig, 1993). Disease transmission is either by direct contact or through the consumption of infected intermediate hosts such as pigeons or earthworms (Redig, 1993).

Chapter 5: General discussion

Hence, captive birds at Wingspan- Birds of Prey Research Centre may have picked up the infection through the direct ingestion of ova or contaminated prey, such as game birds, which they are fed occasionally. Moreover, the birds here are not maintained on routine anthelmintics and only one morepork was administered a topical ectoparasiticidal preparation (Ivomec®, Merial labs) two months before sampling. However, none of the birds carrying capillaria eggs showed clinical signs of the disease, indicating a possible sub-clinical infection that has the potential of mounting to a clinical case of capillariasis if the birds become immune-compromised or during an increase in parasite burden inside the host.

5.2 Scope and limitations of the study

This study has shown that New Zealand falcons, Australasian harriers and moreporks are susceptible to *Plasmodium* spp and *T. gondii* infection, though the pathogenicity of the organisms towards these birds is yet to be understood. Also, the role of these raptors as hosts in the life cycle of the pathogens and their contribution towards disease transmission in New Zealand over the years needs further research before the results can be applied to conservation management of the species.

The finding of the high incidence of capillariasis in both wild and captive raptors will help in better planning of conservation programs, such as the captive breeding and re-introduction of New Zealand falcons, especially in guiding the anthelmintic treatment protocols for captive birds. Attention must be paid towards the effect of captive, re-introduced raptors on other wild birds prevailing in the intended re-introduction site, in terms of transmission of *Capillaria* spp. Additionally, studies can be designed to determine the possibility and routes of capillaria transmission between re-introduced raptors and other birds and vice-versa. Since the pathogenicity of *Capillaria* spp among New Zealand's raptors is not well understood, experimental transmission of the organism to these birds may be designed to better understand the effect of sub-clinical infection on general fitness and post-release survival of the bird and also determine the threshold of parasite burden the host can carry before succumbing to clinical capillariasis.

Though the presence of pathogens like *Plasmodium* spp and *T. gondii* among sampled wild and captive New Zealand raptor populations has been confirmed, an accurate estimate of their prevalence in the country's total raptor populations could not be determined owing to my small sample size. Moreover, many of our sampled birds were those admitted to Wildbase Hospital, Massey University, Palmerston North and our sampling procedure showed an obvious geographic

bias wherein many of the sampled birds were from the nearby Manawatu-Wanganui regions and only a few from the far north or south of the country. This bias is governed by the location of the wildlife hospital in Palmerston North, which receives most birds from the Manawatu- Wanganui area and in no way indicates that the birds in this region are more susceptible to mortalities or contraction of the aforementioned disease than the others. Hence, a more detailed study involving sampling over a larger area needs to be carried out to provide an accurate estimate of the prevalence of the pathogens.

5.3 Future implications of the study

The identification of *Plasmodium* spp and *T. gondii* organisms among New Zealand's raptors has opened up new areas of research, which have direct implication on the health and conservation of these birds, especially the New Zealand falcon. While there was no evidence of clinical disease in this study, this does not preclude that these organisms are having subclinical effects on host fitness and survival. We need to understand the dynamics of avian malaria as a disease process among raptors and the effect of *Plasmodium* spp on the survival rate of nestlings, influence of seasons on disease transmission and correlation between sex and susceptibility to infection, to name a few. For example, a study on the effect of chronic avian malaria infection on the reproductive rate of lesser kestrels revealed that the reproductive success of infected males was lower than non-infected males. But there was no such difference observed among females and this might be attributed to the breeding behavior of raptors, wherein males are more involved in chick rearing than the females and *Plasmodium* spp infections might have hampered their foraging ability (Ortego, et al., 2008). Additionally, parasitaemia can give rise to competition for nutrients with the host and compromise egg production, either due to loss of valuable nutrients to the parasite or its expenditure by the immune system, thereby reducing the reproductive rate of chronically infected adult birds (Marzal et al., 2005). However, it is also possible that chronic *Plasmodium* infections might encourage the evolution of resistant or tolerant birds, provided the affected birds can maintain a stable population size and if resistance to the disease has the potential to be a hereditary trait (Kilpatrick et al., 2006).

If subclinical effects are present in New Zealand raptors, then the time and location for reintroduction of New Zealand falcons might have to be carefully chosen henceforth, keeping in mind the seasonal variations in the abundance of *Plasmodium* spp and vectors in the release sites. A study by Baillie et al. (2012), mapped the abundance of *Plasmodium* spp in source and founder populations of New Zealand bellbirds *(Anthornis melanura*) and observed spikes during winter, autumn and spring season. These seasonal variations were attributed to factors such as competition for food and territory; both of which played the role of

immune-stressors. A similar study may have to be designed to evaluate the influence of seasons on the abundance of pathogens and vectors in the chosen release sites of the falcon and the various immune-stressors prevalent in the area, which might cause recrudescence of disease due to asymptomatic, chronic avian malaria infection.

None of the birds that tested positive for *Plasmodium* spp and *T. gondii* infections in our study showed signs characteristic to either disease, as reported in the post mortem databases so far. Hence, the pathogenicity of both diseases among New Zealand's raptors is virtually unknown. Future studies involving experimental infections of Australasian harriers, moreporks and New Zealand falcons with *Plasmodium* spp and *T. gondii* antigens can be carried out to evaluate their pathogenicity and the immune status of these birds with respect to the former. For example, experimentally induced toxoplasmosis in Crested caracaras *(Caracara cheriway)*, Great horned owls *(Bubo virginianus)*, Barred owls *(Strix varia)* and screech owls *(Asio otus)* through the feeding of infected tissues, produced an increase in *T. gondii* specific IgG antibodies, but without the presence of any clinical signs associated with the disease, allowing the authors to concluded that these species were clinically resistant to toxoplasmosis (Vitaliano et al., 2010; Dubey, et al., 1992)

Therefore, similar studies might be required to analyse the status of resistance and susceptibility of New Zealand's raptors towards *Plasmodium* spp and *T. gondii* infections. If resistant to clinical infections, it might also be important to understand the role of these birds as carriers, in the indirect transmission of both pathogens to other native bird species. Moreover, experimentally induced infections of *Capillaria* spp., *Plasmodium* spp and *T. gondii* would provide information regarding the course of disease, possible routes of transmission in a captive setting, clinical signs associated with the disease (if found to affect the birds clinically) and its prognosis. This information will enable caretakers of raptors to take preventive measures and identify birds that may be suffering from the aforementioned diseases based on the clinical signs displayed by them, if any. Similarly, since the role of raptors in the transmission of these pathogens to other susceptible species is unknown, information gained from experimental studies will provide conservationists with information that might influence the location of re-introduction of endangered raptors like the New Zealand falcon and help understand the role of raptors in the life cycle of infectious pathogens in New Zealand. Experimental studies might be essential for the conservation of New Zealand falcons, since this species could be novel to the aforementioned pathogens and any information regarding diseases of New Zealand falcons is extrapolated from global falcon literature, thereby exposing the falcon to possible extinction in the wake of an epidemic. Understanding the dynamics of a disease process among New Zealand falcons will prepare conservationists to

make informed decisions regarding its release by identifying pathogen infested areas and regions where New Zealand falcons may pose a risk to other susceptible native birds.

Another important avenue for research is the prevalence of co-infections among new Zealand's raptors, as seen in the case of South Island saddlebacks *(Philesturnus carunculatus carunculatus)* suffering from *Plasmodium* spp and avipoxvirus infections (Alley, et al., 2010). Since some of our sampled raptors showed co-infections with *Plasmodium* spp and *T. gondii* and one New Zealand falcon were infected with both *P. elongatum* and *P. relictum* species, it would be interesting to observe whether these co- infections increase the susceptibility of the hosts towards other pathogens and the combined effect of concurrent infections on the general raptor populations of the country.

5.3 References

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APPENDIX 1: TISSUES FROM 117 RAPTORS WERE SELECTED FOR MOLECULAR ANALYSIS TO DETERMINE THE PRESENCE OF *PLASMODIUM* SPP AND *T. GONDII*

A1.1 New Zealand falcons

Accession No	Tissue type[A]	Tissues	Avian malaria	Toxoplasma	Sequencing
49652	P.E	liver, spleen	x	x	
49723	P.E	liver, lung, kidney	x	x	
49914	P.E	liver, lung, kidney	x	x	
50348	P.E	liver, heart, ventriculus	√	x	P. sp SGS1 (JX196866)(99%)
50355	P.E	liver, spleen, intestines	√	x	P. sp GRW6 (DQ68381) (99%)spleen, liver
47950	P.E	liver, lung	x	x	
47951	P.E	lung, liver	x	x	
48029	P.E	liver, lung, brain	x	√	*T. gondii* (U81497)(100%) lung, brain
49157	P.E	lung, liver, spleen	x	x	
46081	P.E	liver, lung	x	x	
46123	P.E	liver, kidney, lung, heart	x	x	
46455	P.E	lung, liver, kidney	x	x	
46552	P.E	lung, liver	x	x	
47115	P.E	liver, lung, kidney	x	x	

Appendix 1: Tissues for retrospective analysis

44747	P.E	liver, lung, muscle	x	x	
44823	P.E	liver, kidney, lung, spleen	x	x	
44933	P.E	lung, liver, kidney, heart	x	x	
45866	P.E	liver, spleen, muscle, kidney	x	x	
43003	P.E	liver, heart, kidney, lung	x	x	
43170	P.E	liver, heart, kidney, lung	x	x	
43339	P.E	liver, heart, kidney, lung, spleen	x	√	T. gondii (U81497)(100%) lung, heart, liver
43471	P.E	liver, lung, kidney	x	x	
43918	P.E	liver, lung, kidney, heart	x	x	
38265	P.E	heart, liver, lungs, spleen	x	x	
39968	P.E	liver, heart	x	x	
40173	P.E	heart, liver, lungs	x	x	
42083	P.E	liver, lung	x	x	
42412	P.E	heart, liver, lungs	x	x	
42862	P.E	heart, liver, lungs	x	x	
32764	P.E	liver, heart, lung	x	x	
33070	P.E	liver, spleen	x	x	
33802	P.E	liver, lung, heart	x	x	
34812	P.E	heart, lung, liver	x	x	
34842	P.E	lung, heart	x	x	

Appendix 1: Tissues for retrospective analysis

36158	P.E	heart, lung, liver	x	x	
Total positives	**35**		**2**	**2**	

A1.2 Australasian harriers

Accession No	Tissue type	Tissues	Avian malaria	Toxoplasma	Sequencing
49771	P.E	liver, heart, lung, spleen?	x	x	
49742	P.E	liver, lung, heart	x	x	
44226	P.E	liver, lung, heart	x	x	
43099	P.E	liver, lung, heart	x	x	
43027	P.E	liver, heart, lung, spleen?	x	√	Insufficient DNA
37972	P.E	liver, lung, heart	x	x	
37587	P.E	liver	x	x	
37391	P.E	lung, liver, heart	x	x	
37389	P.E	lung, liver, heart	x	x	
37376	P.E	lung, liver, heart	x	√	T. gondii (U81497)(100%) lung, liver
50821	F.F	lung, liver	x	x	
49661	F.F	lung, liver	x	x	
49625	F.F	liver, heart, lung	√	x	P. sp GRW6 (DQ68381) (99%)lung, liver
49529	F.F	liver, heart, lung	√	√	P. sp GRW6 (DQ68381) (99%)lung, liver T. gondii (U81497)(100%) lung, liver
49259	F.F	liver, heart, lung	√	√	P. sp GRW6 (DQ68381) (99%)lung, liver

Appendix 1: Tissues for retrospective analysis

			T. gondii (U81497)(100%) lung, liver	
43392	F.F	liver, lung	x	x
46154	F.F	liver, lung	x	x
46159	F.F	liver, lung	x	x
46254	F.F	liver, lung	x	x
46330	F.F	liver, lung	x	x
46361	F.F	liver, lung	x	x
46456	F.F	liver, lung	x	x
46626	F.F	liver, lung	x	x
47324	F.F	liver, lung	x	x
47336	F.F	liver, lung	x	x
47996	F.F	liver, lung	x	x
48132	F.F	liver, lung	x	x
36187	P.E	liver, lung	x	x
36749	P.E	liver, lung	x	x
36774	P.E	liver, lung	x	x
36802	P.E	liver, lung	x	x
36824	P.E	liver, lung	x	x
37155	P.E	liver, lung	x	x
37179	P.E	liver, lung	x	x
37652	F.F	liver, lung	x	x
39676	F.F	liver, lung	x	x
39781	F.F	liver, lung	x	x
43063	F.F	liver, lung	x	x
43072	F.F	liver, lung	x	x
43477	F.F	liver, lung	x	x

Appendix 1: Tissues for retrospective analysis

Accession	Tissue type	Tissues	Avian malaria	Toxoplasma
43478	F.F	liver, lung	x	x
44625	F.F	liver, lung	x	x
44836	F.F	liver, lung	x	x
44937	F.F	liver, lung	x	x
46040	F.F	liver, lung	x	x
46323	F.F	liver, lung	x	x
Total	**46**		**3**	**4**

A1.3 Morepork

Accession No	Tissue type	Tissues	Avian malaria	Toxoplasma	Sequencing
42986	F.F	liver, lung	x	x	
43393	F.F	liver, lung	x	x	
43435	F.F	liver	x	x	
44402	F.F	liver	x	x	
46734	F.F	liver, lung	x	x	
46758	F.F	liver, lung	x	x	
47794	F.F	liver, lung	x	x	
49191	F.F	liver, lung	x	x	
49409	F.F	liver, lung	x	x	
49674	F.F	liver, lung	x	x	
49683	F.F	liver, lung	√	x	*P.* sp. AFTRU5 (DQ847263) (100%) lung, liver
49821	F.F	liver, lung	x	√	*T. gondii* (U81497)(100%) lung, liver
39074	F.F	lung	x	x	
40711	F.F	liver, lung	x	x	
41027	F.F	liver	x	x	

Appendix 1: Tissues for retrospective analysis

41549	F.F	liver	x	x	
41798	F.F	liver, lung	x	x	
41799	F.F	liver, lung	x	x	
41881	F.F	liver, lung	x	x	
43472	F.F	liver, lung	√	x	*P. relictum (*AF254975) (100%) liver, lung
43473	F.F	liver, lung	√	√	P. relictum (AF254975) (100%) liver, lung T. gondii (U81497)(100%) lung, liver
43967	F.F	liver, lung	x	x	
43256	F.F	liver, lung	√	x	P. relictum (AF254975) (100%) liver, lung
43827	F.F	liver, lung	x	x	
25998	F.F	liver, lung	x	x	
29042	F.F	liver, lung	x	x	
33536	F.F	liver, lung	x	x	
34065	F.F	liver, lung	x	x	
35392	F.F	liver, lung	x	x	
35654	F.F	liver, lung	x	x	
35945	F.F	liver, lung	√	x	P. relictum (AF254975) (100%) liver, lung
36000	F.F	liver, lung	x	x	
36423	F.F	liver, lung	x	x	
36454	F.F	liver, lung	x	x	
36905	F.F	liver, lung	x	x	
38954	F.F	liver, lung	x	√	T. gondii (U81497)(100%) lung, liver
Total	**36**		**5**	**3**	

Appendix 1: Tissues for retrospective analysis

APPENDIX 2: NESTED PCR PROTOCOL FOR THE DETECTION OF *PLASMODIUM* DNA IN FORMALIN-FIXED OR PARAFFIN-EMBEDDED TISSUES

Nucleic acid extraction	Kit
DNA	Qiagen Dneasy Blood and Tissue kit.

Primers	Name	Sequence (5'-3')	Size (bp)	Target
Forward	NF1	CATATATTAAGAGAAITATGAG	~600	*Cytochrome b gene*
Reverse	NR3	ATAGAAAGATAAGAAATACCATTC		
Forward	F	ATGGTGCTTTCGATATATGCATG	480	
Reverse	R2	GCATTATCTGGATGTGATAATGGT		

PCR kit: Invitrogen Platinum Taq Polymerase

Reagent mix	First round	Second round
Sterile distilled water	15.35µl	36.7µl
10xPCR	2.55µl	5µl
MgCl$_2$ (50mM)	0.7µl	1.5µl
dNTPs (10mM)	0.8µl	1.6µl
10µM primer	1.5µl (NF1)	2µl (F)
10µM primer	1.5µl (NR3)	2µl (R2)
Taq	0.1µl	0.2µl
DNA	2.5µl	1µl (from First round)
Total volume	**25µl**	**50µl**

Appendix 2: Avian Malaria PCR Protocol

PCR controls	Description
Positive	Previously Sequenced *Plasmodium* positive sample
Negative	Nuclease free water

PCR program name: avian malaria

Cycling parameters: First round	Temp (°C)	Time	No. of cycles
Hold	94	3min	1
Denature	94	30sec	
Anneal	50	30sec	25 (1st round)
Extension	72	45sec	35 (2nd round)
Hold	72	10min	1
	4	hold	

Electrophoresis	Description	Size of amplicon(s) (bp)
Agarose gel	1.5%	480
MW marker	100bp	

Reference

Hellgren, O., Waldenstrom, J., Bensch, S. (2004). A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. Journal of Parasitology 90(4): 797-802.

Appendix 2: Avian Malaria PCR Protocol

APPENDIX 3: DETECTION OF *TOXOPLASMA GONDII* DNA IN FORMALIN-FIXED OR PARAFFIN-EMBEDDED TISSUES

Nucleic acid extraction	Kit
DNA	Qiagen Blood and tissue DNeasy extraction kit

Primers	Name	Sequence (5'-3')	Size	Position	Target
Forward	Food 1	GGAACATCCGCTGAAGCTCATGG	491bp	1169	*Pppk-dhps*
Reverse	Food 2	CAGAGAATCCAGTTGTTTCGAGG		1660	gene
Forward	Food 3	CAGTCCAGACTCGTCACCGATC	400bp	1201	
Reverse	Food 4	CCGGAATAGTGATATACTTGTAG		1616	

PCR kit: Invitrogen Platinum Taq Polymerase

Reagent mix	First round	Second round
Sterile distilled water	17.55µl	38.3µl
10x PCR buffer	2.5µl	5.0µl
$MgCl_2$ (50mM)	0.75µl	1.5µl
dNTPs	0.5µl	1.0µl
10µm primer	0.5µl Food-1	1.0µl Food-3
10 µm primer	0.5µl Food-2	1.0µl Food-4
Taq	0.2µl	0.2µl
DNA	2.5µl	2µl (from first round)
Total volume	**25µl**	**50µl**

PCR controls	Description
Positive	Toxoplasma DNA extracted from *Toxovax*
Negative	Nuclease free water

PCR program name: Toxo food

Cycling parameters: 1st round	Temp (°C)	Time	No. of cycles
Hold	94	5	1
Denature	94	30 sec	
Anneal	57	30 sec	40
Extension	72	1 min	
Hold	72	10 min	1
	4	∞	
Cycling parameters: 2nd round	**Temp (°C)**	**Time**	**No. of cycles**
Hold	94	5 min	1
Denature	94	30 sec	
Anneal	57	30 sec	40
Extension	72	30 sec	
Hold	72	10 min	1
	4	∞	

Electrophoresis	Description	Size of amplicon(s)
Agarose gel	1.5%	400bp
MW marker	100bp	

Reference

Roe, W. D., Howe, L., Baker, E. J., Burrows, L., & Hunter, S. A. (2013). An atypical genotype of *Toxoplasma gondii* as a cause of mortality in Hector's dolphins (*Cephalorhynchus hectori*). *Veterinary Parasitology, 192*(1-3), 67-74.

Appendix 3: Toxoplasma PCR Protocol