Diagnosis and Prevalence of Ventricular Nematodiasis in Kiwi (*Apteryx* spp.)

A thesis presented in partial fulfilment of the requirementsfor the degree of

Masters of Veterinary Science

at Massey University, Manawatū,New Zealand

Bhargavi Chidambaram

2021

Abstract

Only one species of nematode has been formally described from the ventriculus of kiwi (*Apteryx* spp.); a spirurid nematode of the *Cyrnea* genus. However, apart from its morphology, little is known about this parasite. This thesis describes investigations into its pathogenicity, prevalence and diagnosis.

A case series examining four cases of ventricular nematodiasis in kiwi (VNK) highlighted that repeated faecal examinations did not detect the eggs of the ventricular nematodes. Diagnoses were achieved via gastroscopy or post-mortem histological examination of the ventricular wall. These cases illustrated how the lack of an accurate ante-mortem diagnostic technique limits what is known about the parasite responsible for VNK including its pathogenesis, transmission and management.

Four-hundred and fifty samples were collected opportunistically from captive kiwi housing facilities over the course of eight months with the dual aims of refining diagnostic methodology and establishing the prevalence of the *Cyrnea* sp. nematode in captive kiwi faecal samples. These samples were obtained from 167 individual kiwi of four different subspecies across a wide age-range from nine different submission locations.

An ante-mortem test to diagnose VNK was formulated and refined using coprological testing. The most sensitive method of detecting *Cyrnea* ova was centrifugal flotation in 80% zinc sulphate solution but examination of the centrifugal sediment from a 33% zinc sulphate

solution was more accurate in quantifying the number of ova present. Centrifugal sedimentation reduces thetime and effort required for sample processing, making it the efficient choiceThe prevalence of ventricular nematode ova in faecal samples from captive kiwi was found to be 16.77% (95% CI) based on faecal flotation in 80% zinc sulphate solution. Using this method, kiwi aged 4 months or younger and thoseof the North Island brown (*A. mantelli*) species were the most likely to have detectable parasitic infection with *Cyrnea* spp.

This research highlighted the importance of developing an accurate ante-mortem faecal test for the diagnosis of VNK. A viable preliminary test forlaboratory use was developed and the prevalence of infection was examined in captive kiwi through faecal testing.

Acknowledgements

I am grateful to my supervisors without whom this research would not have been possible. My main supervisor Dr Megan Jolly was supportive and encouraging throughout and helped overcome key obstacles encountered. My ourse major leader Prof Brett Gartrell was always available for guidance, especially during the data analysis and initial planning stages. This research is a follow up to the initial investigations performed by Dr Adrienne French whowas kind enough to let me borrow the research questions arising from her thesis and publications as well as serving as my subject expert. Mrs Barbara Adlington introduced and guided me through working in the Massey University Parasitology Laboratory and was a key player in obtaining my researchsamples.

This thesis relied on samples collected from several captive kiwi housesacross the country. This was made easy by the staff at the National Kiwi Hatchery, Pūkaha Mount Bruce, Willowbank Wildlife Reserve, National Aquarium, Kiwis for Kiwis, Wellington Zoo and Wildbase hospital. The post- mortem sampling for this project was made possible by Dr Stuart Hunter. I amgrateful to all these people, not only for providing my samples but also encouraging my efforts and displaying genuine interest in this project.

I am grateful for having had the opportunity to work in the Wildbase department and School of Veterinary Science at Massey University, Palmerston North, and the funding they provided for this

research.

Lastly, I would be remiss if I did not acknowledge the endless support received from my family, friends, colleagues, coffee, and God.

Table of Contents

List of Figures

List of Tables

1 Literature Review

1.1. Introduction

Ventricular nematodiasis has been recognised in birds since 1800 (Herman & Wehr, 1954). It is understood to be responsible for several mass mortalities among waterfowl (Borgsteede et al., 2006; Niemuth et al., 2013) and more recently in captive rheas (*Rhea spp.*) (Ederli et al., 2020). However,in kiwi, (*Apteryx* spp.) it is mentioned only in passing (Anonymous, 1978; Boardman, 1995; Smith et al., 1973). Despite being noted in post-mortem examinations of kiwi, it has been infrequently reported in the scientific literature. This review provides an overview of kiwi biology and conservation management, the information currently available on ventricular nematodiasis in kiwi and how current kiwi management strategies may affect this disease.

1.1. Biology and Captive Management of Kiwi (*Apteryx* spp.) in NewZealand

1.1.1. Overview of Kiwi Biology

Kiwi are casuariiformes endemic to New Zealand (Robertson, 2013). Taxonomically, they belong to the family Apterygidae and the order Apterygiformes (Barlow, 2018). Being

palaeognathic ratites, (Barlow, 2018) they are most phylogenetically similar to emus, rheas and ostriches. There are currently five species of kiwi: little spotted kiwi (*A. owenii*), great spotted kiwi (*A. haasti*), southern brown or tokoeka (*A. australis*), North Island brown (NIB)kiwi (*A. mantelli*) and Okarito brown or rowi (*A. rowi*). These species are furtherdivided into fourteen taxa (Germano et al., 2018) on the basis of genetic distinctiveness and for the purposes of conservation management.

Kiwi are characterised by their nocturnal behaviour, absent tail and vestigial wings. The largest of the species is the great spotted kiwi which can weigh up to 3.3kg. The smallest species is the little spotted kiwi at ~1.3kg. Females are typically larger than males in all species (Heather & Robertson, 2005; Robertson, 2013). Despite differences in their breeding behaviour, (refer table 1.1) all kiwi feed on invertebrates and fallen fruit by probing into the soil with their bills (Heather & Robertson, 2005).

Table 1.1 Ecological characteristics of Kiwi (Apteryx spp.) (Germano et al., 2018; Heather & Robertson, 2005)

Species	**English name**	**Te Reo Māori Name**	**Adult Weight (kg)**	**Breeding Season**	**Incubation behaviour**	**Estimated population size**
A. australis	Southern brown/ tokoeka	Tokoeka, tokoweka, roa	2.2-2.8	June-March	Shared	24850
A. rowi	Okarito brown/ rowi	Rowi	1.9-2.6	July-March	Shared	600
A. mantelli	North Island brown kiwi	Kiwi, kiwi a whenia toaroa	2.4-3.1	July-March	Male only	25100
A. haastii	Great spotted	Roroa, roa	2-4-3.3.	July-December	Shared	14000
A. owenii	Little spotted	Kiwi pukupuku, kukupapata iti	1.1-1.3	September-February	Male only	1900

1.1.2. The problems facing kiwi populations

All kiwi species are either at risk or threatened in terms of conservationstatus (Robertson et al., 2016). This is largely due to habitat loss; genetic bottle-necking and predation from mustelids, cats (*Felis catus*) and dogs (*Canis familiaris*) (Barlow, 2018; Germano et al., 2018)

The effects of predation are the most significant threat to kiwi populations. Mustelid and cat attacks remain the primary cause of declining kiwi populations as they leave very few chicks to survive and breed as adults (Germano et al., 2018). Adult kiwi are much more resistant to attacks from mustelids and feral cats but can be killed by dogs. The loss of a reproductively active adult kiwi is much more damaging that the loss of a juvenile bird (Germano et al., 2018). Thus controlling predatory attacks is the single most important factor in ensuring the survival and growth of kiwi populations (Germano et al., 2018).

There is a tendency for kiwi to develop genetically distinct subpopulations because of the physical distance between adjacent populations. This separation occurs due to several reasons including their flightlessness, limited dispersal power and particular sensitivity to geographicbarriers like rivers and mountains (Germano et al., 2018). Further, the previously distinct subpopulations of the NIB kiwi — Eastern, Western, Northland and mixed origin — are no longer genetically sustainable in captivity due to population bottle-necking

(Barlow, 2018). As a result, most conservation efforts are focused on the Eastern subgroup.

1.1.3. Conservation Efforts

In light of these circumstances, several efforts at conservation of kiwi have been established. These fall under the banner of the Kiwi Recovery Plan which includes the New Zealand Department of Conservation (DOC) and over a hundred independent partners (Germano et al., 2018). The broad strokes ofthis plan include genetic management, captive breeding, artificial incubation of wild eggs and creche rearing of juvenile kiwi (Barlow, 2018; Germano et al.,2018). The incubation of wild eggs is known as Operation Nest Egg (ONE).

Captive breeding of kiwi aims to improve the genetic makeup of wild populations. This is especially important given that the ability of a population to resist and fight infections, depends on its heterozygosity (Barlow, 2018). Genetic management also aims to prevent gene loss and maximize translocation and ONE successes (Germano et al., 2018). In order to achievethis in kiwi, founder genes are artificially selected. Captive breeding is carriedout in birds that are minimally related to each other. This is done with the aim of creating offspring that express rare alleles and have as much heterozygosityas possible. The parent birds are captured from the wild or are reared throughONE (Germano et al., 2018). For captive kiwi, genetic management also involves breeding only genetically pure Eastern brown kiwi and gradually phasing out other

subpopulations (Barlow, 2018). This use of genetic information in breeding and management is essential to ensure the future viability of kiwi populations (Germano et al., 2018).

Predation of kiwi is, as mentioned, a concerning issue. This is dealt withusing predator trapping programmes. These came into vogue in the 1960s andhave been the major method of pest-eradication since then (Germano et al.,

2018). A major driving force for predator control is the Predator Free New Zealand 2050 (PF2050) project. While not specifically focussed on kiwi, this project brings together several groups of stakeholders and DOC staff to ensure that native wildlife can flourish without the threat of predation by introduced mammals. It is led by the DOC and the Predator Free NZ Trust (Predator Free Trust, 2020). The current methods employed include the use of toxins and ground-based stoat-control technologies. Aerial application of pest control poisons such as para-aminopropiophenone (PAPP) and 1080 have been demonstrated to be effective methods of protecting kiwi in the NorthIsland (Germano et al., 2018). Kiwi aversion training for pet dogs is an educational tool that is showing positive results, but corresponding education of dog owners remains lacking. Therefore, interagency cooperation is requiredto bring about a change in the common practices and culture of dog owners. Simultaneously, DOC staff must also be educated about collecting suitable evidence to ensure the prosecution of offending dogs (Germano et al., 2018). In addition, the creation and maintenance of pest-free

islands and mainland sanctuaries are important to provide kiwi a safe environment to which they may be relocated. Continuing support of pest-free locations is also highly important to ensure adequate predator control (Germano et al., 2018).

Operation Nest Egg was developed as a method to rapidly increase thepopulation of endangered kiwi. It was started in 1994 and focusses on crecherearing of young kiwi away from the dangers of predators (Germano et al., 2018). Kiwi eggs or chicks are removed from the wild and hatched and/or reared in captivity till they are big enough to thwart predator attacks. This is

usually when they reach a weight of 1000g (Bassett, 2012). The juveniles, so raised, are returned to the wild. ONE increases the chance of survival of kiwi chicks from 5% (if raised in the wild) to 65%. It has had the most success in NIB kiwi as the techniques used were honed on this species (Bassett, 2012).

1.1.4. Successes of Kiwi Conservation Management

Conservation management of kiwi has halted the annual 2% populationdecline, especially of the three rarest species: little spotted, rowi and Haast tokoeka. (Germano et al., 2018). It is predicted that by 2030, these efforts willhave resulted in a 42% increase in the overall kiwi population spread over all the five species (Germano et al., 2018). This, however, will require management of very small populations as well as predator and landscape control (Germano et al., 2018).

1.1.5. Issues and Limitations of Captive Management

Captive management, despite being our best line of defence against population loss and extinction, is fraught with difficulties. In terms of genetic variability, it has been estimated that at least 90% of wild founder kiwi must be transferred to a single management facility to sustain adequate genetic diversity (Barlow, 2018). Further, the focus on the Eastern brown kiwi with a planned "phasing out" of the Western, Northland and mixed varieties (Barlow, 2018) prioritises the Eastern brown kiwi at the cost of other subpopulations/genotypes. The more immediate concern remains the lack of suitable breeding females; both in terms of age and genetic makeup (Barlow,2018). This lack of genetic diversity is most apparent in little spotted kiwi as
the population has been expanded from only five founder individuals (Germano et al., 2018).

The success of ONE has been limited with great spotted kiwi and Haasttokoeka due to their social structure and easily-stressed nature. Further, this method of intensive management increases the risk of disease (Robertson, 2013).

Captive management of kiwi, both for breeding and rearing of juvenile birds, results in kiwi being held in confined pens or creches. The same groundis often repeatedly used for many years with successive batches of kiwi. This can result in the development of health issues and diseases that are not seencommonly in wild kiwi, where the population

density is much lower.

1.2. An Overview of Common Diseases of Kiwi

Kiwi are affected by several diseases, both infectious and non-infectious. Infectious diseases are bacterial, viral, fungal or parasitic in origin. Examples of bacterial diseases reported in kiwi include tuberculosis (*Mycobacterium avium*) and erysipelas (*Erysipelothrix rhusiopathiae*). Enterobactericeae such as *Salmonella* spp., *Yersinia enterocolitica* and *Escherichia coli* have been isolated from cases of bacterial enteritis in captiveand wild kiwi (Morgan, 2008). *Clostridium* spp. and *Campylobacter* spp. havealso been isolated from kiwi but their clinical significance is still undeterminedand these may be commensal flora of the gastrointestinal tract (Morgan, 2008). A variety of Gram positive and negative bacteria have been implicatedin cases of rhinitis, pneumonia and air sacculitis (Alley & Gartrell, 2019). Viraldiseases of kiwi are less frequently reported with avian pox being the most commonly reported such condition (Alley & Gartrell, 2019). A circovirus has been isolated from rowi but its clinical significance is unknown (White et al., 2016). Fungal diseases of kiwi are usually of the respiratory tract with aspergillosis being the most pathogenic. Cryptococcosis has also been reported. Candidiasis can occur in immunosuppressed individuals as a secondary infection (Alley & Gartrell, 2019). Parasitic diseases of kiwi are discussed in section 1.3.1.

Kiwi commonly suffer from several non-infectious diseases.

These include foreign body ingestion which can result in gastric impaction and/or traumatic gastritis. Cloacal prolapse and impaction have also been reported. The former may be a sequela to normal oviposition. Dystocia or egg binding and other disease of the reproductive tract also occur. These conditions can result in salpingitis and metritis. These may also develop secondary to air sacculitis or pneumonia (Morgan, 2008). While these are typically diseases ofadults, neonatal/juvenile kiwi are susceptible to yolk sac retention and yolk sacculitis. The latter may be aseptic or associated with septicaemia (Morgan, 2008). These conditions are thought to be associated with artificial incubation parameters. Juveniles may also suffer congenital angular limb deformities of the tibiotarsi and splay legs (Morgan, 2008) which are thought to be at least partially associated with over-feeding induced rapid growth in captive-reared birds.

There are several additional factors that can compound the severity of disease in kiwi. Creche management is associated with pathogen multiplication and a high degree of stress, which can suppress immunefunction and consequently increase susceptibility to disease and parasites

(Alley & Gartrell, 2019; Germano et al., 2018). The decline in genetic diversityand founder alleles in kiwi populations have been suggested to undermine theability of kiwi to resist infection (Barlow, 2018). This is further complicated by the indirect effects disease have on kiwi and wildlife populations. These include a hampered ability of the host to

forage, inability to fight predators and immunosuppression (Alley & Gartrell, 2019). These secondary effects are especially significant in kiwi given their propensity for predatory attacks and allelic homozygosity. Lastly, the limited past research into these diseases hashandicapped conservationists and veterinarians in effectively understanding and managing them. These concerns hold particularly true for parasitic diseases.

1.2.1. Parasitic Diseases of Kiwi

The paucity of information regarding diseases of New Zealand wildlife has been established. This is all the truer for parasitic diseases — not just of New Zealand birds but wild aves worldwide. Several factors contribute to this including incomplete baseline information regarding the hosts, the difficulty ofsampling them and the often-limited number of hosts present in the wild (Wobeser, 2008).

This is especially true for kiwi and much of our knowledge comes from understanding disease processes in other species, including parasitic infections. The loss of host resources exerted by the parasite through direct pathogenic effects as well as competition with the host for nutrients, cost to host immunity and loss of growth potential are all fundamental to parasitism (Wobeser, 2008). Though the extent of these effects vary with each parasite

and host, they have been demonstrated in kiwi with coccidial infections (Morgan et al., 2012).

There have been twenty-one different endoparasites reported from kiwi,but several have been taxonomically categorized only to the genus level (Morgan et al., 2012). Of these, three are haemoprotozoa such as avian malaria (Banda et al., 2013). The bulk of the remaining parasites are helminthsof the gastrointestinal tract (Alley & Gartrell, 2019; Morgan et al., 2012). Thesehelminths are also reported to cause visceral and neural larval migrans (French, 2021). The most well-studied and pathogenic parasitic disease of kiwiis coccidiosis. It is the single most limiting disease factor to creche rearing of young kiwi (Morgan, 2013).

The signs associated with gastrointestinal parasitism in kiwi are nonspecific such as anorexia, weight loss, diarrhoea and sometimes death (Morgan et al., 2012). Diagnosis of most infections is achieved through faecalexamination and treatment is through the use of standard anthelminthic and antiprotozoal medications (Morgan et al., 2012). The use of routine coprological screening (Taylor et al., 2019) of captive kiwi allows for rapid diagnosis, treatment and control of endoparasitism.

Ventricular nematodiasis in kiwi has been infrequently reported. Very little information, including the exact aetiology, is known about this condition. However, the aetiology is thought to be a spirurid of the *Cyrnea* genus from the family Habronematidae (French, 2021). While the number of reported post-mortem cases has been increasing in recent years, recognition of clinical infections in live birds is rare (French, 2021). A likely reason for this is the lackof an appropriate diagnostic test. This also makes it difficult to distinguish

clinical cases of ventricular nematodiasis from other causes of gastritis, especially as the clinical signs are vague.

1.3. Avian Spirurid infections

The order Spirurida is made of two suborders — Spirurina and Camallanina (Bowman, 2021). Early taxonomic classification was incredibly complex with the order composed of several families dispersed among many superfamilies (Dunn, 1978). There remain many superfamilies of clinical veterinary significance. These include Gnathostomatoidea, Physalopteroidea,Spiruroidea, Habronematoidea, Thelazioidea, and Filariroidea, ((Bowman,2021). Most of these genera occur in fish and bird hosts (Dunn, 1978).

Morphologically, there is great diversity among the various genera of Spirurida. However two features are common to all species belonging to this order — a rhabditiform larval stage and a flat, tightly coiled spiral tail in males (Deplazes et al., 2016; Dunn, 1978). Females are also typically larger than males. Other common characteristics include the presence of two to four pseudolabia or lips (except for Thelaziidae). These surround an often sclerotised vestibule instead of a true buccal capsule. The oesophagus is clearly divided into an anterior muscular portion and posterior, larger glandularportion. Males bear a pair of caudal alae and spicules, the latter of which mayor may not be equal in length. Caudal papillae, while present in most species,vary greatly in number and position. It is these differences that aid species identification

and classification (Deplazes et al., 2016; Dunn, 1978).

Spirurid nematodes that inhabit the upper gastrointestinal tract are usually found in the fore-stomachs of ruminants or the proventriculus and ventriculus of birds. They occur both in the lumen and burrowed in the mucosaof these organs (Dunn, 1978).

The lifecycle of these nematodes is always indirect (Dunn, 1978). Mostspirurids are oviparous though few are viviparous or ovo-viviparous. The eggs are typically small, thick-shelled, embryonated and often elongated (Dunn, 1978). These are laid by the gravid female within the host's gut, excreted in the faeces and soon hatch into the first larval stage. This larval stage must beingested by an intermediate host or paratenic host. These are often arthropodsor maggots, but their exact species or even order, among avian hosts, remain unknown (Dunn, 1978; Zhang et al., 2011). Once the first larval stage is ingested by the intermediate host, it develops into its third larval stage. This isusually the stage that is infective to the final host (Dunn, 1978).

1.4. Ventricular nematodiasis in kiwi

1.4.1. Aetiology

Ventricular helminthiasis is caused by several genera of parasites in several genera of aves. Among waterfowl, trichostrongylids of the *Amidostomum* and *Epomidiostomum* genera, spirurids like *Streptocara* and cestodes such as *Gastrotaenia* are common (Choe et al., 2016;

Friend & Franson, 1999; McLaughlin & McGurk, 1987; Nowicki et al., 1995; Zhang et al., 2004). In domestic species and passerines, the common causative agentsinclude *Hadjelia* spp. and *Cheilospirura* spp. (Blakeney & Dimmick, 1971; Ederli & de Oliveira, 2014; Friend & Franson, 1999; Ganapathy & Sharma, 2003; Herman & Wehr, 1954; Naem et al., 2012; Razmi et al., 2007). Among

ratites, the parasites causing ventricular nematodiasis are usually habronemes of the genera *Cyrnea* and *Procyrnea* (Ederli & de Oliveira, 2019; Zettermann et al., 2005). These parasites have also been documented in raptors, keas and passerines (David & Lindquist, 1982; Ebmer et al., 2020). Inkiwi there is little information regarding nematodes responsible for parasitiases and much less on those affecting the ventriculus specifically (Yeates et al., 2012).

The first and only nematode formally identified from the ventriculus of kiwi is a spirurid designated as *Cyrnea apterycis* (Harris, 1975; Yeates et al., 2012). The genus *Cyrnea* was proposed to account for habronemes of avian origin. It was recently further divided into separate genera *Procyrnea, Metacyrnea* and *Cyrnea* based on morphological differences (Niemuth et al., 2013; Zhang et al., 2004). Recent studies on the gastrointestinal parasites of kiwi indicate that the commonly found ventricular worm morphologically resembles *C. apterycis* (French, 2021) or a closely related species. This remains our current best understanding of the parasites responsible for ventricular nematodiasis in kiwi (VNK).

The nematodes of the genus *Cyrnea* display morphology in keeping with related spirurids and genera (such as *Procyrnea*). The adult female worms measure between 4-6mm in length and 100-300 *μ*m in diameter. The males are smaller by up to 1.5 times (Ederli et al., 2020; French, 2021). Amphids and phasmids with deirids behind the nerve rings as well as cuticularstriations may be present. The buccal capsule is characterised by two bilobedlabia (located dorsally and ventrally) as well as pseudolabia. The entire buccal capsule is laterally flattened (Bagnato et al., 2018; Clark, 1978; Ederli & de Oliveira, 2019; French, 2021; Zhang et al., 2011). Typical of spirurida is a muscular-glandular type oesophagus with a clear distinction between the twoportions (Taylor et al., 2016). The male tail is tightly coiled in a spiral. Males also have well developed caudal alae and two spicules of unequal length (French, 2021). The left spicule is usually longer that the right (Zhang et al., 2004). In the female, the tail ends in a blunt, conical tip. The ovaries are didelphic, uteri opisthodelphic and vulva prodelphic. Each uterus may hold upto 200 eggs which are embryonated, small (50-55 x 25-30 *μ*m), asymmetrical,ovoid with a thick smooth wall and two opercula (Bagnato et al., 2018; Ederli & de Oliveira, 2019; French, 2021; Niemuth et al., 2013; Zhang et al., 2004).

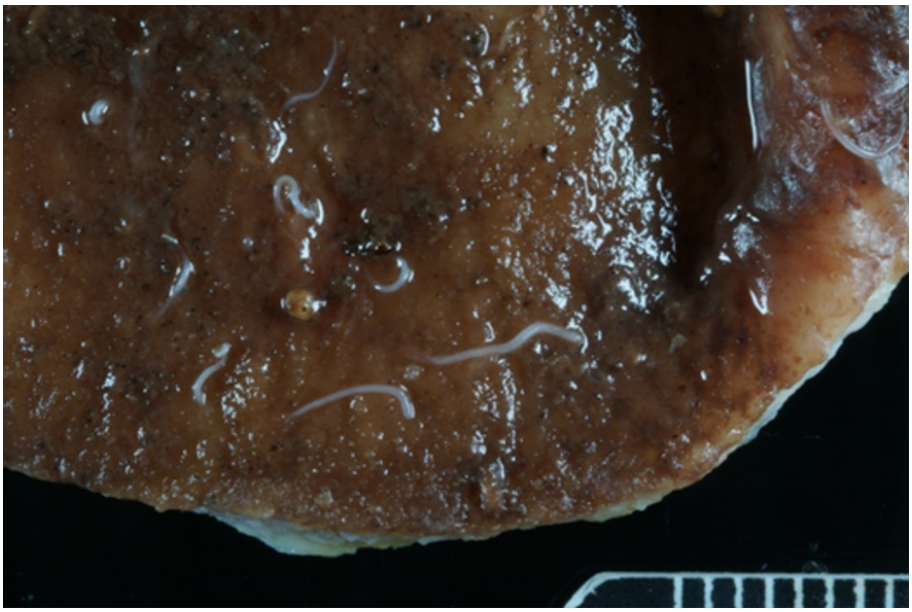

Figure 1.1 Adult *Cyrnea* sp. worms found in the ventriculus of a kiwi (*Apteryx* sp.) during post mortem. The worms occur both in the ventricular lumen and embedded in the koilin layer

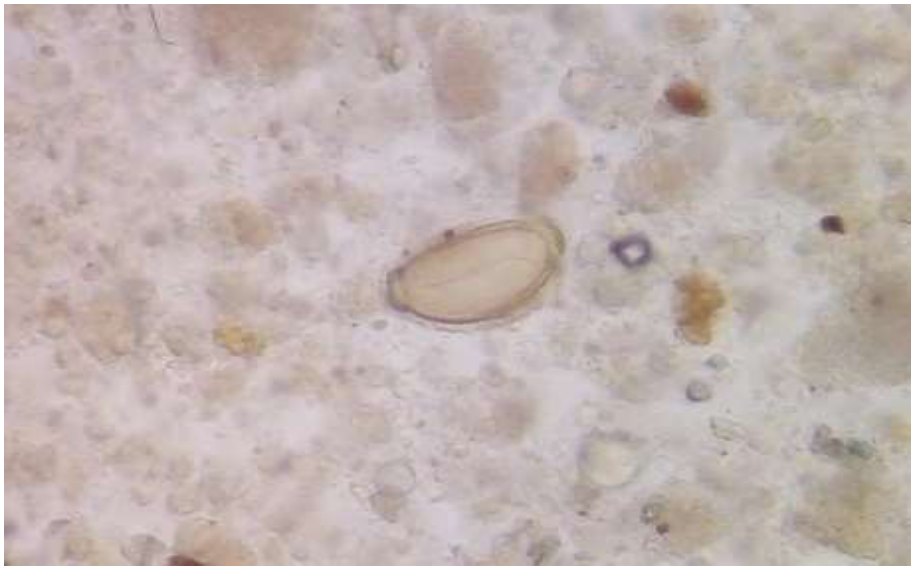

Figure 1.2 Microscopic (40x) appearance of *Cyrnea* sp. ova revealing the asymmetrical shape, opercula and yellow-brown colour

1.4.2. Origins of the parasite

New Zealand has a long history of deliberate introduction of invasivespecies (Lymbery et al., 2014). The country harbours at least 30 mammals,

34 birds, 2000 invertebrates and 2200 plants that have been naturalised (Norton, 2009). Invasive parasites have been introduced along with these hosts and this pathway of introduction has been recognized globally as a major threat to natural biodiversity through spillover infections (Lymbery et al., 2014).Invasive species have often contributed to the loss (and sometimes extinction)of natural biota and their long-term impacts are still under investigation (Norton, 2009). The question of whether kiwi ventricular nematodes are introduced is important because a non-host adapted parasite is generally thought to cause more significant pathology. Further, if an introduced host isa reservoir for this parasite, then exposure of kiwi populations, both wild and captive, may be difficult to control. As parasites and hosts co-evolve, it is expected that hosts develop resistance, resilience or tolerance to the parasites (Moller & Erritozoe, 1996).

In order to establish the alien nature o a pathogen, several criteria mustbe met. The first is the presence of the natural host of the parasite in the new environment. While parasites have been itroduced to new populations through paratenic or intermediate hosts, intrduction through the final host is much more favourable to long-term parasite

establishment (Lymbery et al., 2014). Further, the dependence on an intermediate host for lifecycle completion may be a major limiting factor to lien invasion (Lymbery et al., 2014). The possible reasons could include the difficulty of the parasite to complete its lifecycle in a new environment and absence of natural hosts for the alien species (Lymbery et al., 2014). Another consideration should be thenatural behaviour and host-specificity of the parasites. None of these factors are known about kiwi ventricular nematodes and their lifecycle is only partiallyunderstood. The most reliable evidence comes from the study of related parasites and hosts. The ventricular nematodes of the rhea (*Rhea americana)* are believed to be host specific (Zettermann et al., 2005). While this information pertains to a related host species and likely a closely related parasite, it does not necessarily mean the same applies to kiwi or any other hosts within the same taxonomic order. There, however, remain additional factors to consider. The presumptive indirect lifecycle of the parasitein kiwi is one of them. Lastly, the geographic isolation of New Zealand with itsunique avian life may contribute to the endemic nature of local parasites. For these reasons, the author considers it more likely for the ventricular nematodes of kiwi to have evolved with their hosts rather than reflecting another invasive species in New Zealand.

Reuse of nests by bird hosts has been shown to result in parasite accumulation and increase the probability of horizontal transmission (Moller &Erritozoe, 1996). Given the current conservation strategy of creche rearing ofyoung, immunologically-naïve kiwi in the same site for

several years, this parasite multiplication can be expected within kiwi creches. In fact, it has beendemonstrated with kiwi coccidiosis (Morgan, 2013). Like coccidiosis, ventricular nematodiasis is presumed to follow a faecal-oral transmission mode (French, 2021). The intermediate hosts, though yet unidentified, are likely to be in the immediate vicinity of the host to propagate infection. As the environment of a captive kiwi remains largely unchanged till their release, there must be a near-constant exposure to the same pathogens and parasites as those of the kiwi previously habiting the same creche. In the absence of regular environmental disinfection and substrate replacement, this would allow parasite multiplication and environmental loading with eggs.

This environmental parasite loading is exacerbated by the ease of horizontal parasite transmission. Even though the lifecycle of this parasite is not fully known, based on the involvement of an intermediate host, the mode of transmission is believed to be horizontal. There is no evidence to suggest vertical transmission of the parasite in kiwi. This information is significant because of the hypothesis that horizontal transmission of parasites is selected evolutionarily to allow increased parasite virulence (Moller & Erritozoe, 1996).This is based on reduced host exploitation by horizontally transmitted parasites in contrast to those transmitted vertically. Horizontally transmitted parasites do not usually debilitate their hosts to the point of impaired host reproduction. This means the host is left with sufficient resources for the parasites to exploit. This ensures that the parasites can continue to multiply and transmit. In

contrast, vertically transmitted parasites can debilitate their hosts so severely and quickly that they run themselves out of host reserves to exploit. This hinders the ability of the parasite to reproduce which in turn limitstheir own transmission (Moller, 1997; Moller & Erritozoe, 1996). The mode of transmission is therefore a major factor in the evolution of parasite virulence (Moller & Erritozoe, 1996).

1.4.3. Parasite Lifecycle and Epidemiology

While there have been considerable strides forward in understanding the taxonomy and morphology of kiwi spirurids, the lifecycle of avian habronemes is still unknown. Based on knowledge about spirurid lifecycles inother host species, it is assumed that *Cyrnea* has an indirect lifecycle involvingarthropods like beetles or crustacea as intermediate hosts. The eggs are laid by adult females when they are in the first larval stage and become infective

in the third larval stage when they are typically ingested by the host (Christie et al., 2011; Mejia-Fava et al., 2013; Niemuth et al., 2013; Zettermann et al., 2005; Zhang et al., 2011). However, the exact intermediate host is unknown. This means that the lifecycle has not been fully elucidated, even in an experimental setting, let alone in naturally occurring conditions.

Given these gaps in our understanding of this disease, it follows that there is little known about its epidemiology as well. Based on initial investigations (French, 2021) and extrapolation from other bird species

(Blakeney & Dimmick, 1971; Friend & Franson, 1999; Nowicki et al., 1995) anage-resistance to the parasites seems to exist, i.e., the most severe infectionsare seen in juvenile and subadult kiwi. Juveniles can become infected within two weeks of hatching (Herman & Wehr, 1954). Adults are thought to suffer less intense infections because of improved overall immunity (French, 2021). Regardless, there exists considerable evidence that VNK is more severe in juvenile rather than adult kiwi.

Ventricular nematodiasis in aves is fairly common. It has been found that up to 77.7% of duck (McLaughlin & McGurk, 1987) and 100% of Canada geese (*Branta canadensis*) and common eider (*Somateria mollissima*) populations (Borgsteede, 2005; Nowicki et al., 1995) can be affected. It is also the most common parasitic infection among captive rheas in Brazil (Zettermann et al., 2005). Most of the work in determining theprevalence in kiwi comes from a retrospective study of necropsy records obtained from the School of Veterinary Science Pathology service, Massey University. It revealed that among NIB with nematodiasis, 16.6% had ventricular nematodes detected histologically at routine post-mortem examination. This study also showed that 22.2% of little spotted kiwi (*Apteryx*

owenii) and 28.6% of rowi kiwi (*Apteryx rowi*) were affected with ventricular nematodes (van Zyl, 2014). However, a prospective study specifically investigating the presence of gastrointestinal nematodes revealed that up to 94% of the examined kiwi were affected, with the most common location, i.e.,84%, being the ventriculus (French, 2021). It

is clear that routine post-mortemexamination and histology massively underestimate the true prevalence of infection in comparison to a detailed parasitological exam. These initial findings further suggest that despite being infrequently reported in kiwi, the true prevalence of ventricular nematodes may be much greater.

1.4.4. Clinical and Pathological Findings of Ventricular Nematodiasis

Ventricular helminth infections are characterized by nonspecific clinicalsigns and pathological changes to the ventriculus. Signs such as inappetence, anaemia, emaciation, perching low and a fluffed-up appearance have been reported. However overwhelming infections can cause peracute death without overt signs (Avelar Ide et al., 2014; Herman & Wehr, 1954; Mejia-Fava et al., 2013; Niemuth et al., 2013). On necropsy, the nematodes are typically found under the koilin and can bury deep within the mucosa (Herman & Wehr, 1954;Nowicki et al., 1995; Zettermann et al., 2005).As a result, much of the intestinal contents obtained at postmortem may be free of both nematode eggs and worm fragments However, the ventriculus of affected birds are often grossly affected owing the aggressive damage induced by the adult worms as they bury beneath the koilin layer. Macroscopic lesions include the lining and koilin of the ventriculus sloughing/eroding and becoming thickened, desquamated, irregular, friable or cheesy. Haemorrhage, ulcers, andmigratory tracts may be seen in the mucosa with nematodes buried as far as the submucosa and muscularis. A granulomatous reaction can develop in

response on the serosal surface and cause coelomic adhesions (Avelar Ide etal., 2014; Ederli & de Oliveira, 2014; Ferrell et al., 2009; French, 2021; Friend

& Franson, 1999; Niemuth et al., 2013; Zettermann et al., 2005). Histologically, a lymphocytic inflammation of the lamina propria with eosinophils, macrophages and multinucleated giant cells is typical (French, 2021).

These changes are due to several reasons. *Cyrnea* spp. and other spirurids tend to burrow through the koilin into the ventricular mucosa (Niemuth et al., 2013; Smith et al., 1973). The nematodes can also cause pathogenic effects by creating an impaction of the proventriculus and ventriculus. There is resultant ingesta spoilage and the pain associated with impaction can trigger anorexia and consequent malnourishment. The latter may also result from the competition for nutrients to the host from the nematodes (Borgsteede, 2005; Ederli & de Oliveira, 2014; Nowicki et al., 1995;Zettermann et al., 2005). It is thus clear that even though epidemics of ventricular nematodiasis are infrequent, infection can still result in severe debility and occasionally death in the host.

1.4.5. Ante-mortem Diagnosis

As with other parasitic infections, ante-mortem diagnosis of ventricular nematodiasis is mainly achieved through coprological examinations, although gastroscopy can also be used in suspected

cases. The common methods arediscussed below with an emphasis on the principles involved. The methodology of these techniques is reviewed more thoroughly in Chapter 3.

1.4.5.1. General Principles of Coprological Examination

Coprological examination is a relatively low-cost technique with moderate sensitivity and specificity. It allows the laboratory detection of eggs,

oocysts, cysts and larvae of parasites (Soares et al., 2020). Every coprologicalexamination technique begins with the faecal sample. This can be obtained byseveral means: collection per rectum/cloaca from the host animal, off theground sampling or a pooled sample from several animals such as those in aflock or herd. It is recommended that no less than 5g of sample volume shouldbe collected per microscopic examination involving a concentration step (Thienpont et al., 1986). While this is simple enough with mammalian species, obtaining this volume is often difficult in small avian patients. In such cases,pooled sampling or simply using less-than-ideal sample volumes is necessary. Once collected, the faecal sample must be examined within 24 hours. This is required to prevent the sporulation or embryonation of the parasite elements. Failing this, the samples can be stored between 2-4^{o}C in a refrigerator. The faeces can also be mixed with 10% formalin to preserve anyparasite elements present (Thienpont et al., 1986).

There are four basic coprological examination procedures: direct

smear, centrifugal sedimentation, centrifugal flotation and spontaneous flotation (Soares et al., 2020). The microscopic examination of prepared samples should be undertaken first under low magnification as this is sufficientto detect most parasites (Thienpont et al., 1986).

1.4.5.2. Direct Faecal Smears

This technique involves placing a pinhead volume of faeces onto a glass slide and adding a drop of water or normal saline to it. Once thoroughlymixed, a coverslip is added, and the slide is examined under low magnification.Using a drop of Lugol's iodine instead of water or saline provides a yellow stainto parasite eggs and protozoa.

This technique, in addition to being extremely simple and quick, is useful to detect nematode larvae and motile protozoa like *Giardia* spp. It is however relatively insensitive compared to other techniques and it cannot provide a quantitative measurement of the parasite ova/eggs being shed and consequently, the severity of infection (Thienpont et al., 1986)

1.4.5.3. Spontaneous and Centrifugal Faecal Flotation

Faecal flotation is a technique of parasite concentration within a given sample to allow detection of parasites within small volumes of faeces. It also allows for diagnosis when the parasite burden is low (Soares et al., 2020). Itis based on the principle of differing specific gravities between the parasitic elements and various liquid media. The

specific gravities of parasite elements are typically more than 1.000. However, if they are suspended in a solution ofhigher specific gravity, they will float to the surface. Such a solution can be prepared by mixing an amorphous salt and water in a specific ratio (Thienpontet al., 1986). The denser a parasite element is, the more concentrated the flotation solution will have to be. This is exemplified by trematode eggs whichare typically high in density and must be floated in solutions of specific gravityexceeding 1.200. The specific gravity of the solutions is checked using a densimeter or hydrometer (Thienpont et al., 1986). This is because the same concentration of different solutions may not translate to equal specific gravities. This difference stems from the difference in densities of the salts used. The solution used must therefore be decided based on the parasite to be detected.

Once the appropriate solution has been prepared, a measured quantityof faeces (such as 0.5-1.0g) is mixed with the solution in a standard 15ml testtube. The faeces may be run through a mesh or sieve to limit the amount of debris mixing with the flotation solution. This not only provides a cleaner float,but also prevents the eggs and oocysts from being obscured by unwanted debris (Thienpont et al., 1986).

A coverslip is then placed over the surface of the liquid. If the sample isallowed to stand without disturbance, the parasites float to the surface in approximately 15 minutes (Thienpont et al., 1986). This is the principle of spontaneous flotation (Soares et al., 2020). Alternately, the sample can be centrifuged at 314g for five minutes. This is the method

of centrifugal flotation(Soares et al., 2020). While centrifugation does not in any way alter the sensitivity of the flotation test, it does reduce the time required to complete it. The parasites can then be detected by examining the coverslip under low magnification.

While this method can be modified to float any parasite by simply usinga denser flotation solution, highly concentrated flotation solutions tend to distort the parasite elements by their osmotic action. It is however the methodof choice for benchtop diagnoses and parasitology labs due to its sensitivity and wide applicability. While expensive and harmful chemicals such as potassium iodomercurate are sometimes used, (Thienpont et al., 1986) theseissues can be avoided by using simple solutions made with saturated sugar orcommon table salt.

1.4.5.4. Faecal Sedimentation

Centrifugal faecal sedimentation makes use of centrifugal force toseparate the parasites from remaining faecal material (Soares et al., 2020). It involves washing and filtering the faecal sample and then suspending it in a sedimentation solution. This processed sample is then centrifuged for a specific time and force. This forces the heavier parasites such as trematode eggs to the bottom of the test tube along with some faecal matter in the form of a pellet. The supernatant is discarded, and the sediment is then examined under a low magnification light microscope.

The first such method, the Teleman method, was developed using

ether and hydrochloric acid (Thienpont et al., 1986). Several modifications of the technique have since been made both in terms of the solutions used and the number of times the faeces is strained/filtered as well as the material used forthis filtration (Soares et al., 2020). The more modern versions use formal-etheras a concentrating solution, ether and ethyl acetate (Manser et al., 2016). As with flotation solutions, the kind used for sedimentation varies greatly. The time and force of centrifugation are also based on the centrifuge available (Manser et al., 2016). Regardless of the variations present, this centrifugal sedimentation is typically used to isolate and identify any heavy parasites such as trematode eggs. As a corollary, it is relatively insensitive to detecting lighter parasites such as protozoal cysts and oocysts. However, it remains a commonly used technique for its simplicity and low cost, as well as higher sensitivity than faecal flotations for dense parasites (Thienpont et al., 1986).

1.4.5.5. Coprological Diagnosis of Ventricular Nematodiasis

The standard method used for examination of kiwi faeces for parasite elements is faecal flotation in 33% zinc sulphate solution (spg 1.18-1.20). However, for several years, this test has failed to reliably detect the eggs of the *Cyrnea* sp. (French, 2021). Reasons for this may be a higher than averageegg density (due to a lack of a perivitelline space) or the fragile nature of habronematid eggs (French, 2021;

Niemuth et al., 2013). Another reason could be that active infections in kiwi are caused by immature worms which are incapable of producing eggs. No coproscopic technique would detect the parasites in such a situation.

Regardless of the reason why kiwi spirurid eggs are not detected in routine coproscopy, the result remains that ante-mortem diagnosis of this disease is incredibly difficult. It is for this reason that kiwi nematodiasis has been so infrequently reported in the past and remains largely an unknown entity.

1.4.5.6. Other methods of diagnosis

A diagnosis of ventricular nematodiasis can also be reached during necropsy examinations if worms are found in the ventriculus during either gross or histological examination (Friend & Franson, 1999). However, as mentioned earlier, histology is a poorly sensitive technique with only a 57.5%detection rate. Furthermore, it does not necessarily reflect the gross burden within the ventriculus (French, 2021).

The other method for ante-mortem diagnosis is gastroscopy of a clinicalpatient. However, the size of the scope available and the patient body weight

can limit its application. For example, the endoscope used in Wildbase hospital, School of Veterinary Science, Massey University is a 7.9 mm diameter speciality gastroscope (Karl-Storz) which can be accommodated in a kiwi weighing at least 800g (Jolly, personal

communication, 2020) Further, the procedure requires anaesthesia, which limits its use as a screening tool and acutely ill patients may not be sufficiently stable to survive the anaesthesiarequired for this procedure.

It is clear that coprological and non-coprological diagnostic methods have consistently failed at reliably detecting VNK. Further, they will continue to do so unless an alternative viable and accurate diagnostic method is developed.

1.5. Treatment and Control of Ventricular Nematodiasis

Captive kiwi institutions make it a common practice to carry out routinefaecal monitoring of kiwi for coccidiosis and helminthiases — usually on aweekly basis. Captive rearing of kiwi is, in fact, sustainable only due to strictparasitic control and routine anti-coccidiosis treatments (Taylor et al., 2019).This emphasis on coccidiosis control is because it is considered the majorlimiting factor in successful creche rearing of juvenile kiwi (Taylor et al., 2019).While there is currently no information available regarding the clinical prevalence of VNK, it is a distinct possibility that this infection is occurring incaptive facilities at higher rates than would be expected in wild chicks. Thenecessity of an intermediate host to complete the lifecycle of the parasitewould imply that the intermediate host must live in reasonably close proximityto the final host. With a high stocking density of young, susceptible kiwi, reared on the same substrate home to the intermediate hosts, it is easy for kiwi chicks to become infected. The only method currently available to

prevent infections is implementing regular anthelminthic dosing even though there is no information available currently regarding the drug of choice (Avelar Ide et al., 2014; Ederli & de Oliveira, 2014; Niemuth et al., 2013; Zettermann et al., 2005). Additionally, environmental management including regular substrate changes and reduced stocking density are essential to ensure the continuing success of captive management programmes when considering coccidiosis (Taylor et al., 2019) and is likely as valuable to mitigation of other enteric parasites. However, in the kiwi management programme, environmental controls are not typically used as most kiwi are housed in planted pens and large creches that are difficult to manage intensively. In these locations, complete substrate change is not considered feasible which makes instigating significant environmental controls in the kiwi management programme logistically difficult.

1.6. Questions for future research

The one possible benefit to having such little knowledge about VNK is that there is much potential for research. The need for an accurate diagnostic test has already been established. Additionally, understanding the ecology and behaviour of the parasite could potentially refine treatment regimens, including identifying periods when treatment is needed and for the assessment of the efficacy of anthelmintics. Understanding the pathogenicity of kiwi ventricular nematodes would also be of value. For example, some ventricular

nematodes of other genera have been demonstrated to feed on the host's

blood (Borgsteede, 2005). Such effects lead to not only hypovolaemic anaemia but also hypoproteinaemia — two of the most severe clinical signs reported. However, the ventricular nematodes of the rhea, another ratite, do not feed on the hosts' blood (Zettermann et al., 2005). Understanding which of these situations occur in kiwi can be made possible only through researching this nematode and the disease it produces.

1.7. Research Objectives

The aim of the research conducted for this thesis was:

A. to elucidate the difficulties in obtaining an accurate antemortem diagnosis by providing an overview of common clinical and post-mortem presentations of VNK encountered at Wildbase Hospital, School of Veterinary Science (SoVS), Massey University and their management
B. to develop a method of diagnosis of VNK through coprological examination by
a. establishing a method of faecal examination using zinc sulphate, sucrose, sodium nitrate and magnesium sulphate solutions of varying concentrations and specific gravities to reliably detect spirurid ova in faecal samples submitted to the commercial pathology service from institutions around New Zealand.

b. post-mortem examination of the ventriculus and caudal intestinal contents of all kiwi submitted to the Wildbase Pathology Service between November 2020 and August 2021 for the presence of adult worms and eggs
c. determining if a correlation exists between the worm burden in the ventriculus and the egg load in the faeces

C. to determine the prevalence of VNK using coprological examination and examine the effects of age, sex, habitat type and location on parasite burden.

1.8. References

Alley, M. R., & Gartrell, B. D. (2019). Wildlife diseases in New Zealand: recent findings and future challenges. *N Z Vet J, 67*(1), 1-11. doi:10.1080/00480169.2018.1520656

Anonymous. (1978). *Whangarei Animal Health Laboratory Report*. Retrieved from

Avelar Ide, O., Almeida, L. R., Santos, H. A., Lima Wdos, S., Lara, L. B., & Ecco, R. (2014). Sicarius uncinipenis and Deletrocephalus cesarpintoi in captive greater rheas of Minas Gerais State, Brazil. *Rev Bras Parasitol Vet, 23*(3), 355-359. doi:10.1590/s1984-29612014053

Bagnato, E., Frixione, M., Digiani, M. C., & Cremonte, F. (2018). A new species of Procyrnea (Nematoda: Habronematidae) parasitic in Rhea pennata (Aves: Rheidae) from Patagonia, Argentina, with a key to species of the genus. *J Helminthol, 92*(4), 504-513. doi:10.1017/S0022149X17000657

Banda, M. E., Howe, L., Gartrell, B. D., McInnes, K., Hunter, S., & French, N. P. (2013). A cluster of avian malaria cases in a kiwi management programme. *N Z Vet J, 61*(3), 121-126. doi:10.1080/00480169.2012.736130

Barlow, S. (2018). *Captive management plan for kiwi*. Australia: Zoo and Aquarium Association 2018

Bassett, S. (2012). *Operation nest egg incubation and chick rearing best practice protocols*. Wellington: Oralis Limited

Blakeney, W. C., & Dimmick, R. W. (1971). Gizzard and intestinal helminths of bobwhite quail in Tennessee. *The Journal of Wildlife Management, 35*(3), 4.

Boardman, W. (1995). *Causes of mortality in North Island brown kiwis at Auckland Zoo 1960-1994*. Paper presented at the Wildlife Society of the NZVA 1994 Seminar Proccedings.

Borgsteede, F. (2005). The gizzard worm, Amidostomum acutum (Lundahl, 1848) Seurat, 1918 in common eiders (Somateria mollissima L.) in the Netherlands. *Helminthologia, 42*, 4.

Borgsteede, F., Kavetska, K., & Zoun, P. (2006). Species of the nematode genus Amidostomum Railliet and Henry, 1909 in aquatic birds in the Netherlands. *Helminthologia, 43*(2), 98-102. doi:10.2478/s11687-006-0019-8

Bowman, D. D. (2021). Helminths. In *Georgis' parasitology for veterinarians* (11 ed., pp. 135-260). USA: Elsevier.

Choe, S., Kim, H., Lim, J., Lee, D., Park, H., Jeon, H.-K., Kim, H., Kim, Y., & Eom, K. S. (2016). New Record of Schistorophus cirripedesmi (Nematoda: Acuariidae) from a Bar-Tailed Godwit, Limosa lapponica baueri (Charadriformes: Scolopacidae) in Korea. *The Korean Journal of Parasitology, 54*(3), 349-355. doi:10.3347/kjp.2016.54.3.349

Christie, J., Schwan, E. V., Bodenstein, L. L., Sommerville, J. E. M., & van der Merwe, L. L. (2011). The sensitivity of direct faecal examination, direct faecal flotation, modified centrifugal faecal flotation and centrifugal sedimentation/flotation in the diagnosis of canine spirocercosis. *Joiurnal of the South African Veterinary Association, 82*, 6.

Clark, W. C. (1978). Procyrnea keasp. nov. (Habronematidae: Spirurida: Nematoda) from the New Zealand Kea (Nestor notabilisGould, 1865) (Aves: Psittaciformes). *Journal of the Royal Society of New Zealand, 8*(3), 323-328. doi:10.1080/03036758.1978.10429384

David, E. D., & Lindquist, W. D. (1982). Determination of the Specific Gravity of Certain Helminth Eggs Using Sucrose Density Gradient Centrifugation. *The Journal of Parasitology, 68*(October 1982), 4.

Deplazes, P., Eckert, J., Mathis, A., von Samson-Himmelstjerna, G., & Zahner, H. (2016). Order Spirurida. In *Parasitology in veterinary medicine* (1 ed., pp. 346-356). The Netherlands: Wageningen Academic Publishers.

Dunn, A. M. (1978). Superfamily Spiruridoea. In *Veterinary helminthology* (2 ed., Vol.

1, pp. 69-72). Glasgow: Butler & Tanner ltd.
Ebmer, D., Wiedermann, S., & Sattmann, H. (2020). Morphological identifications of gastrointestinal nematodes and acanthocephalans of raptors and owls from Austria. *Annalen des Naturhistorischen Musems in Wien, B, 122*, 6.
Ederli, N. B., & de Oliveira, F. C. R. (2014). Macroscopic lesions of the ventriculus of Rhea americana , Linnaeus, 1758 (Aves: Rheidae) naturally infected by Sicarius uncinipenis (Molin, 1860) (Nematoda: Habronematidae). *J Parasitol, 100*(6), 860-863. doi:10.1645/13-416.1
Ederli, N. B., & de Oliveira, F. C. R. (2019). Redescription of Procyrnea uncinipenis (Molin, 1860) (Nematoda: Habronematidae) based on material from Rhea americana (L.) (Aves: Rheidae). *Syst Parasitol, 96*(8), 735-745. doi:10.1007/s11230-019-09880-w
Ederli, N. B., Gallo, S. S. M., & de Oliveira, F. C. R. (2020). Natural infection by Procyrnea uncinipenis (Nematoda, Habronematidae), a parasite from rheas, an autoctone bird from South America, in emus Dromaius novaehollandiae, a ratite from New Zealand. *Sci Rep, 10*(1), 16541. doi:10.1038/s41598-020-73754-1
Ferrell, S. T., Pope, K. A., Gardiner, C., Bradway, D. S., Ambrose, D. L., MacLean, R. A., Norton, T. M., Stedman, N. L., & Garner, M. M. (2009). Proventricular nematodiasis in wrinkled hornbills (Aceros corrugatus). *Journal of Zoo and Wildlife Medicine, 40*, 8.
French, A. (2021). *A prospective survey of gastrointestinal nematodes in kiwi (Apteryx spp.).* (PhD Thesis). Massey University, Palmerston North, New Zealand.
Friend, M., & Franson, J. C. (1999). Gizzard worms. In E. A. Ciganovich (Ed.), *Field manual of wildlife diseases feneral field procedures and diseases of birds* (pp. 438): U.S. Department of the interior
U.S. Geological survery.
Ganapathy, K., & Sharma, R. S. K. (2003). Cheilospirura species (gizzard worm) infection in battery-caged commercial layer chickens. *Veterinary Record, 153*, 2.
Germano, J., Barlow, S., Castro, I., Colbourne, R., Cox, M., Gilles, C., Hackwell, K., Harawira, J., Impey, M., Reuben, A., Robertson, H. A., Scrimgeour, J., Sporle, W., & Yong, S. (2018). *Kiwi Recovery Plan 2018-2028*. (64). Wellington: Department of Conservation
Harris. (1975). Two New Nematodes Parasitic in the Kiwi in New Zealand. *Bulletin of the British Museum*, 7.
Heather, B. D., & Robertson, H. A. (2005). Kiwi. In *The field guide to the birds of New Zealand* (pp. 18, 19, 167-169). Auckland: Penguin Books.
Herman, C. M., & Wehr, E. E. (1954). The occurrence of gizzard worms in Canada geese. *The Journal of Wildlife Management, 18*(October), 5.
Jolly, M. (2020). Gastroscopy of kiwi birds. In B. Chidambaram (Ed.), (pp. 1).
Lymbery, A. J., Morine, M., Kanani, H. G., Beatty, S. J., & Morgan, D. L. (2014). Co-invaders: The effects of alien parasites on native hosts. *Int J Parasitol Parasites Wildl, 3*(2), 171-177. doi:10.1016/j.ijppaw.2014.04.002
Manser, M. M., Saez, A. C., & Chiodini, P. L. (2016). Faecal Parasitology: Concentration Methodology Needs to be Better Standardised. *PLoS Negl Trop Dis, 10*(4), e0004579. doi:10.1371/journal.pntd.0004579
McLaughlin, J. D., & McGurk, B. P. (1987). An analysis of gizzard worm infections in fall migrant ducks at Delta, Manitoba, Canada. *Canadian Journal of Zoology, 65*, 8.
Mejia-Fava, J., Divers, S. J., Jimenez, D. A., Ambrose, D. L., Rech, R., Gottdenker, N. L., & Mayer, J. (2013). Diagnosis and treatment of proventricular nematodiases in an umbrella cockatoo. *Journal of American Veterinary Medical Association, 242*, 5.
Moller, A. P. (1997). Parasitism and the evolution of host life history. In D. H. Clayton

& J. Moore (Eds.), *Host-parasite evolution: General principles and avian models* (pp. 105-127). Oxford: Oxforn University Press.
Moller, A. P., & Erritozoe, J. (1996). Parasite virulence and host immune defense: host immune response is related to nest reuse in birdds. *Evolution, 50*(5), 7.
Morgan, K. J. (2008). *Kiwi first aid and veterinary care*. Wellington: Department of Conservation.
Morgan, K. J. (2013). *Coccidiosis in the kiwi (Apteryx spp.) : aspects of the pathology, epidemiology and parasite biology : a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Veterinary Science at Massey University, Palmerston North, New Zealand.* (Doctor of Philosopy (Ph.D.). Doctoral). Massey University, Retrieved from http://hdl.handle.net/10179/6340
Morgan, K. J., Alley, M. R., Pomroy, W. E., Castro, I., & Howe, L. (2012). Enteric coccidiosis in the brown kiwi (Apteryx mantelli). *Parasitol Res, 111*(4), 1689-1699. doi:10.1007/s00436-012-3008-5
Naem, S., Houston, R. S., & Sentíes-Cué, G. (2012). New insights into morphological features of Hadjelia truncata (Spirurida: Habronematidae), as revealed by SEM. *Parasitology Research, 112*(1), 327-333. doi:10.1007/s00436-012-3140-2
Niemuth, J. N., Allgood, J. V., Flowers, J. R., De Voe, R. S., & Troan, B. V. (2013). Ventricular Habronemiasis in Aviary Passerines. *Case Reports in Veterinary Medicine, 2013*, 1-6. doi:10.1155/2013/719465
Norton, D. A. (2009). Species invasions and the limits to restoration: Learning from the New Zealand experience. *Science, 325*(July), 4.
Nowicki, A., Roby, D. D., & Woolf, A. (1995). Gizzard nematodes of Canada geese wintering in southern Illinois. *Journal of wildlife disease, 31*(3), 7.
Predator Free Trust. (2020). Predator free 2050 vision. *The big picture.* Retrieved from https://pf2050.co.nz/the-predator-free-movement/
Razmi, G. R., Kalidari, G. A., & Maleki, M. (2007). First report of the Hadjelia truncata infestation in pigeons of Iran. *Iranian journal of veterinary research, 8*(19), 4.
Robertson, H. A. (2013, 2017). North island brown kiwi. *New Zealand Birds Online.*
Robertson, H. A., Baird, K., Dowding, J. E., Elliott, G. P., Hitchmough, R. A., Miskelly, C. M., McArthur, N., O'Donnell, C. F., Sagar, P. M., & Scofield, R. P. (2016). *Conservation status of New Zealand birds*.
Smith, B. L., Poole, W. S. H., & Martinovich, D. (1973). Pneumoconiosis in the captive new zealand kiwi. *Veterinary Pathology, 10*, 8.
Soares, F. A., Benitez, A. D. N., Santos, B. M. D., Loiola, S. H. N., Rosa, S. L., Nagata, W. B., Inacio, S. V., Suzuki, C. T. N., Bresciani, K. D. S., Falcao, A. X., & Gomes, J. F. (2020). A historical review of the techniques of recovery of parasites for their detection in human stools. *Rev Soc Bras Med Trop, 53*, e20190535. doi:10.1590/0037-8682-0535-2019
Taylor, H. S., Morgan, K. J., Pomroy, W. E., & McInnes, K. (2019). Apparent lack of efficacy of toltrazuril against Eimeria species affecting brown kiwi (Apteryx mantelli) at a captive rearing facility. *N Z Vet J, 67*(2), 101-104. doi:10.1080/00480169.2018.1541425
Taylor, M. A., Coop, R. L., & Wall, R. L. (2016). Veterinary parasitology, 4th Edi. In: Wiley Blackwell, UK.
Thienpont, D., Rochette, F., & Vanparijs, O. F. J. (1986). *Diagnosing helminthiasis by coprological examination* (Vol. 1986): Janssen Research Foundation Beerse, Belgium.
van Zyl, N. (2014). *Nematodiasis and larval migrans in kiwi (Apteryx spp.) : a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Zoology at Massey University, Palmerston North, New Zealand.* (Master of Science (M.Sc.) Masters). Massey University, Retrieved from http://hdl.handle.net/10179/6322
White, D. J., Hall, R. J., Wang, J., Moore, N. E., Park, D., McInnes, K., Gartrell, B. D.,

& Tompkins, D. M. (2016). Discovery and complete genome sequence of a novel circovirus-like virus in the endangered rowi kiwi, Apteryx rowi. *Virus Genes, 52*(5), 727-731. doi:10.1007/s11262-016-1342-x
Yeates, G. W., Zhao, Z. Q., Hitchmough, R. A., & Stringer, I. A. N. (2012). The conservation status of New Zealand Nematoda. *New Zealand Entomologist, 35*(2), 128-130. doi:10.1080/00779962.2012.686317
Zettermann, C. D., Nascimento, A. A., Tebaldi, J. A., & Szabo, M. J. (2005). Observations on helminth infections of free-living and captive rheas (Rhea americana) in Brazil. *Vet Parasitol, 129*(1-2), 169-172. doi:10.1016/j.vetpar.2004.12.015
Zhang, L., Brooks, D. R., & Causey, D. (2004). Procyrnea Chabaud 1958 (Nematoda: habrnonemtoidea: habronematidae) in birds form the area de conservacion guanacaste, Costa Rica, including desciptions of 3 new species. *Journal of Parasitology, 90*, 10.
Zhang, S., Song, J., & Zhang, L. (2011). Three species ofProcyrneaChabaud, 1958 (Nematoda: Habronematoidea: Habronematidae) from raptors in Beijing, China, with descriptions of two new species. *Journal of Natural History, 45*(47-48), 2915-2928. doi:10.1080/00222933.2011.622451

2 Ventricular Nematodiasis in Kiwi (*Apteryx* sp.): A Case Series

2.1. Abstract

Four cases of ventricular nematodiasis in kiwi (VNK) were examinedin a case series using two ante-mortem and two post-mortem cases. All the reported cases were from captive North Island Brown kiwi (*Apteryx mantelli)* from various institutions located across New Zealand. The ante- mortem cases were admitted to Wildbase Hospital, Massey University, forloss of weight, anaemia and diarrhoea with melaena/haematochezia. Initialdiagnostic tests in the form of survey radiography and faecal examination revealed ventricular impaction and enteric coccidiosis. Treatment for theseconditions did not result in resolution of clinical signs, prompting further diagnosis using gastroscopy. This provided a diagnosis of VNK. Both cases were treated with anthelminthics. The first case had a positive patient outcome while the second case resulted in patient mortality. The post-mortem examination of the second patient confirmed severe VNK both on gross and histopathological examinations. The koilin of the ventriculus was damaged and a mononuclear inflammatory reaction was observed. These findings were also noted in the post-mortem cases (cases3 and 4). While the third case was euthanized for unrelated reasons, the fourth bird was determined to have succumbed to VNK.

All the cases occurred in juvenile kiwi reared in captive institutions.In both ante-mortem cases, routine faecal examinations did not detect theeggs of the parasite responsible, and a diagnosis was reached only after the patients failed to improve in hospital. Further, two of the cases discussed resulted in host mortality and all caused severe morbidity. For these reasons, developing a suitable and appropriate diagnostic techniquefor accurate detection of VNK should be considered a priority.

2.2. Introduction

The North Island brown kiwi (NIB) (*Apteryx mantelli*) is a ratite native toNew Zealand. This species has lost much of its population from a combination of habitat loss and predation by introduced mammals such as mustelids, catsand dogs (Robertson, 2013). In order to combat these threats, captive conservation management and rearing programmes such as Operation Nest Egg (ONE) were started. Eggs are collected from the wild and incubated in captivity. The hatched kiwi are hand reared and released when they attain the weight of one kilogram to reduce predator-caused morbidities and mortalities (Taylor et al., 2019). These measures have more than proven their benefit to kiwi populations. They have doubled the numbers of rowi kiwi (*A. rowi*) and helped shift the NIB kiwi from a “threatened” conservation category to “declinings”(Robertson et al., 2016). Another benefit to captive rearing is that it has allowed us to

better understand and expand our knowledge of the diseases affecting managed species (Alley & Gartrell, 2019). While this is true for manykiwi diseases, helminthiases remain poorly understood. This is especially true for helminths specific to the ventriculus. The nematodes in question are still not fully identified and described. However, one or more species, from the genus *Cyrnea*, are thought to be responsible (French, 2021; Harris, 1975; Yeates et al., 2012). Ventricular worms have been identified during routine necropsy of kiwi for several years, yet they have not often been associated with clinical disease due to a lack of characteristic ante-mortem clinical signs (Hunter, personal communication, 2020). However, recent studies suggest that these parasites may be a more significant issue than previously thought (French, 2021). This case series aims to provide more evidence in support of this hypothesis by reporting on two clinical cases and two post-mortem casesof VNK.

2.3. Case Descriptions

2.3.1. Case 1

A juvenile NIB kiwi of unknown sex was admitted to Wildbase Hospital, SoVS, Massey University for inappetence and melaena. Radiographs taken prior to admittance by the referring veterinarian had shown the presence of several stones in the ventriculus and gas accumulation in the intestines. Initialphysical examination revealed that stones in the ventriculus were readily palpable and that the

patient was thin with a body condition score of 3.5 out of9, weighing 778g. The patient was initially treated with butorphanol 4mg/kg (Butorgesic 10mg/ml Ilium) intramuscular, twice daily and fluid therapy using Hartman's solution (Baxter) was administered both as 8ml orally, (~10ml/kg) twice daily and 10ml (~15ml/kg) subcutaneously twice daily.

Further radiographic examination showed that in addition to the stonesin the ventriculus noted previously, a metallic foreign body was also present. Haematological and biochemical analyses revealed leucocytosis (23.4 $x10^9$/l (RR: 8.7-14.5 $x10^9$/l (Morgan, 2008))). On faecal flotation there were 25,000 coccidial oocysts per gram (OPG). The initial diagnosis included gastric impaction and coccidiosis. The leucocytosis was suspected to be a sign of an unknown infection or related to the coccidiosis. Consequently, the patient wastreated with toltrazuril 20mg/kg (Baycox 50mg/ml, Bayer) orally, once daily forthree days, trimethoprim-sulphamethoxazole 20mg/kg (Deprim 240mg/5ml,AFT Pharmaceuticals) orally, once daily for three days and amoxycilin-clavulonate 125mg/kg (Curam 250+62.5mg/5ml, Novartis) orally twice dailyalong with the fluid therapy and analgesia that had already been initiated.Three grams of psyllium husk (Metamucil 3.4g/tablespoon) was added to theoral fluids to aid in flushing out the ventricular stones and metallic foreign body.Radiographs repeated the following week showed that the metallic foreign body had passed through the gastrointestinal

system but theventricular stones remained. The coccidiosis burden was reduced but hadn'tfully resolve after treatment (8750 OPG). The patient's weight and appetitefluctuated daily. Gastroscopy was performed to further investigate these signs. The patient was anaesthetised using isoflurane (Attane 250ml, Bayer) inoxygen at 4% for induction and maintenance and a flexible endoscope with 7.9 mm outer diameter (Karl Storz Endoskope) was used to examine the upperGIT. Gas insufflation of the ventriculus revealed that the mucosa had multiplehaemorrhagic ulcers. The ventriculus was then flushed with normal saline. This allowed visualization of several white-translucent nematodes within the lumen (Figure 2.1). Some of these were free floating but most were partially embedded in the ventricular wall. The patient was diagnosed with VNK.

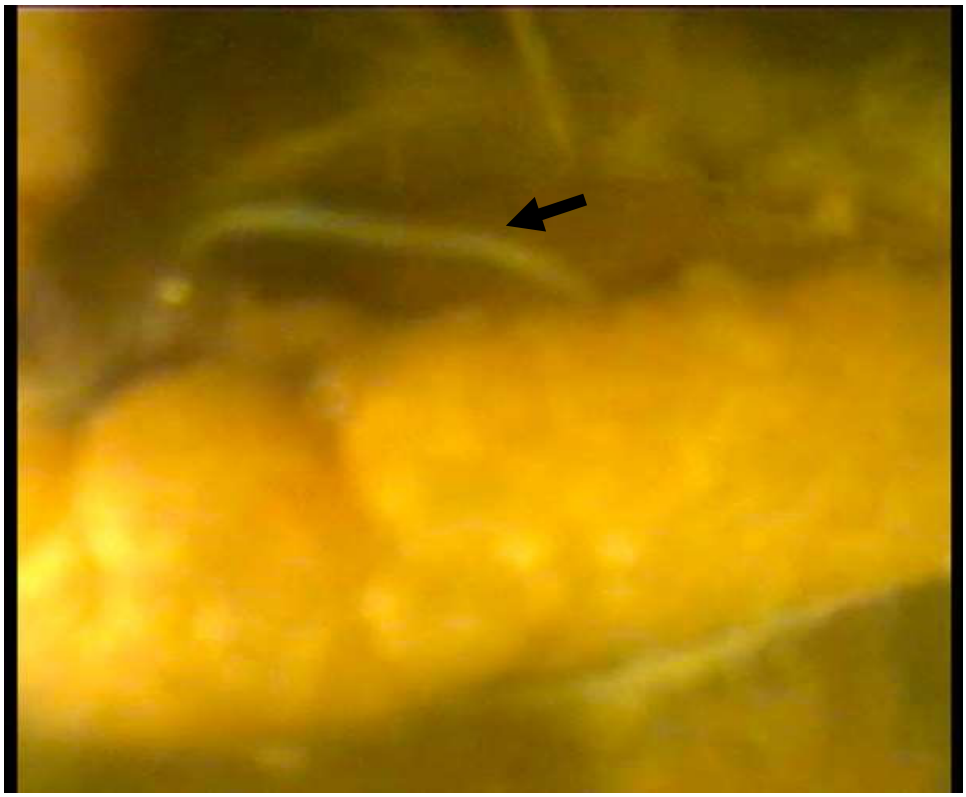

Figure 2.1 Endoscopic view of the ventriculus of a brown kiwi (*Apertyx mantelli*) showing

a *Cyrnea* sp. nematode partially embedded in the koilin layer (Case 1)

Consequently, sucralfate 25mg/kg (Carafate 1g tablets Aspen) orally twice daily was added to the treatment regimen. Butorphanol analgesia was replaced with tramadol 15mg/kg (Tramadol 10mg/ml Biomed) orally twice daily. Additionally, fenbendazole 25mg/kg, (Panacur, 100mg, Merck) orally, once daily for five days and moxidectin 0.2mg/kg, (Cydectin 1g/l Zoetis) in a one-off dose, orally were used to control the helminth infection.

After ten days of treatment, the patient's weight and appetite improved.Repeat radiographs showed that the rest of the ventricular stones werepassing through the intestines. Faecal examination was negative for coccidial oocysts and nematode eggs. A second gastroscopy was performed using theprocedure mentioned above to confirm the efficacy of anthelminthic treatment.It revealed a marked reduction in the ulceration and erythema of the ventricular wall. The patient was discharged six weeks after admission to a captive rearing facility and was released to the wild a month later, having met all growth and health targets.

2.3.2. Case 2

A juvenile, male NIB kiwi was referred to Wildbase Hospital for inappetence and severe weight loss one month after release into a monitored wild population. The patient weighed 937g on

presentation. No other clinical signs were noticed on initial physical examination. The patient was initially treated with butorphanol 4mg/kg (Butorgesic 10mg/ml Ilium) intramuscular, twice daily and fluid therapy using Hartman's solution (Baxter) 20ml orally, (~20ml/kg) twice daily. Survey radiographs taken the following day revealed many stones in the ventriculus. Faecal flotation detected 18,900 coccidial OPG of faeces and an uncounted number of tapeworm ova. Haematological and biochemical analyses indicated panhypoproteinaemia (8g/L (RR:54-62g/L(Morgan, 2008))) and high levels of creatinine kinase (1339 U/L (RR: 521-971 U/L (Morgan, 2008))). The patient was diagnosed with malnourishment, coccidiosis and ventricular stone impaction. Consequently, toltrazuril, trimethoprim-sulphamethoxazole and psyllium husk were added to the treatment regimen in the same doses used in case 1.

After a week of improvement in appetite and demeanour, the patient became anorexic and started to lose weight. It also suffered occasional boutsof regurgitation and produced soft, melaenic faeces. The bird became severely hypoproteinaemic and anaemic with heterophilic leucocytosis (Heterophils: 28.35 $x10^9$/l (RR: 4.0-8.2 $x10^9$/l (Morgan, 2008))). A blood transfusion was performed with 10ml blood collected from a healthy NIB kiwi donor. Additionally, 10ml hydroxyethyl starch (Voluven, 6%, Fresenius Kabi Norge AS) was administered intravenously slowly over an hour. The patient was placed on 2.5% dextrose solution, (Baxter, 1l) intravenously as a

continuous rate infusion (CRI) of 2.7ml/kg/hour. Amoxycillin-clavulonate 125mg/kg (Curam 250+62.5mg/5ml, Novartis) intravenously, twice daily, was also administered for five days.

Gastroscopy (using the same method as in case 1) was performed to investigate possible causes of intraluminal blood loss that might explain the patient's anaemia. This revealed ulceration of the ventricular mucosa and several white-translucent worms at the isthmus. The procedure wasterminated before examination of the ventriculus was completed due to anaesthetic instability of the patient. The patient was subsequently treated for the ventricular ulceration, potentially caused by nematodiasis, with sucralfate 25mg/kg (Carafate 1g tablets Aspen) orally twice daily and moxidectin 0.2mg/kg (Cydectin 1g/l Zoetis) orally, in a one-off dose, in addition to the supportive care and antibiotic therapy mentioned previously.

Despite these efforts, the patient was found dead ten days after initial admission.

Gross post-mortem examination revealed congestion and oedema of the lungs. The ventriculus was markedly distended with sloughing of the koilin layer. Histopathology revealed that the ventriculus contained several adult andlarval nematodes that were either in viable or degenerative condition with associated mononuclear and granulocytic inflammation of the proventricular glands. The lungs were found to have no inflammatory response

despite fluid present in some air capillaries. The pathologist determined that the most likelycause of death was aspiration of fluid and that the patient had been severely affected by VNK.

2.3.3. Case 3

An eight-week-old, female NIB kiwi weighing 420g was presented to Wildbase Hospital, Massey University for anorexia, haematochezia and a stumbling gait. Initial physical examination determined that the patient was dehydrated and pain was elicited on manipulation of the left stifle joint. The bird was initially treated with dextrose 2.5% (Baxter) intravenously, administered as a CRI at 2.4ml/kg/hr. The patient was also provided with butorphanol analgesia at 4mg/kg (Butorgesic 10mg/ml Ilium) intramuscularly, twice daily and antibiotic therapy with amoxycillin-clavulanate 125mg/kg (Curam 250+62.5mg/5ml, Novartis) intravenously, twice daily. The following day, survey radiographs were taken under gaseous anaesthesia. Isoflurane gas (Attane 250ml, Bayer) in oxygen was used at 4% for induction and maintenance. The radiographs revealed severe rotational deformity of the left tibiotarsus. As a result, the prognosis was poor for release to the wild. The patient was euthanised with pentobarbital 300mg/kg (Pentobarb 300, 300mg/ml, Provet) intravenously.

Gross necropsy findings showed that the patient was in poor body condition with reduced body fat reserves. The ventriculus

contained a small amount of pasty khaki material and grit. The intestines were devoid of digestatill the level of the caecae which contained clear watery-to-mucoid fluid mixed with brown, pasty material. The entire intestinal tract was turgid and the serosal vessels were injected.

Histology revealed several adult nematodes buried in the mucosa of the ventriculus beneath the koilin. They were surrounded by proteinaceous material, necrotic debris, macrophages and a few eosinophils. There were also several lymphoid aggregates and lysed erythrocytes within the lamina propria. The intestines displayed increased mononuclear cells and heterophils. The pathological diagnosis was of severe VNK and enteritis in combination with severe rotational deformity of the left tibiotarsus.

2.3.4. Case 4

A six-week-old, male, NIB kiwi was presented dead to the Wildbase Pathology service, SoVS, Massey University for necropsy. Camera footage from his captive institution revealed that he had been active the previous day.External examination of the carcass showed that the animal was in good bodycondition, weighing 456g, and had adequate fat reserves and muscle mass. Gross necropsy findings showed that the ventricular koilin was partially damaged - friable, dull, granular and loosely adhered to the underlying mucosa. The intestines were mostly empty throughout their length till the level

of the cloaca, containing little digesta and gas. Histopathology of the ventriculus revealed several mature and immature nematodes within themiddle and deeper layers and also the glandular junction of the ventriculus. Anumber of these parasites were buried in the mucosa and submucosa. A mononuclear inflammatory reaction predominated by epithelioid and multinucleated giant cells was seen around some nematodes. The deep lamina propria and submucosa were, however, predominated by a marked lympho-plasmocytic inflammation. Necrotic debris and degenerate nematode fragments were also seen scattered through the glands (Figure 2.2). Based on these findings, the cause of death was determined to be VNK.

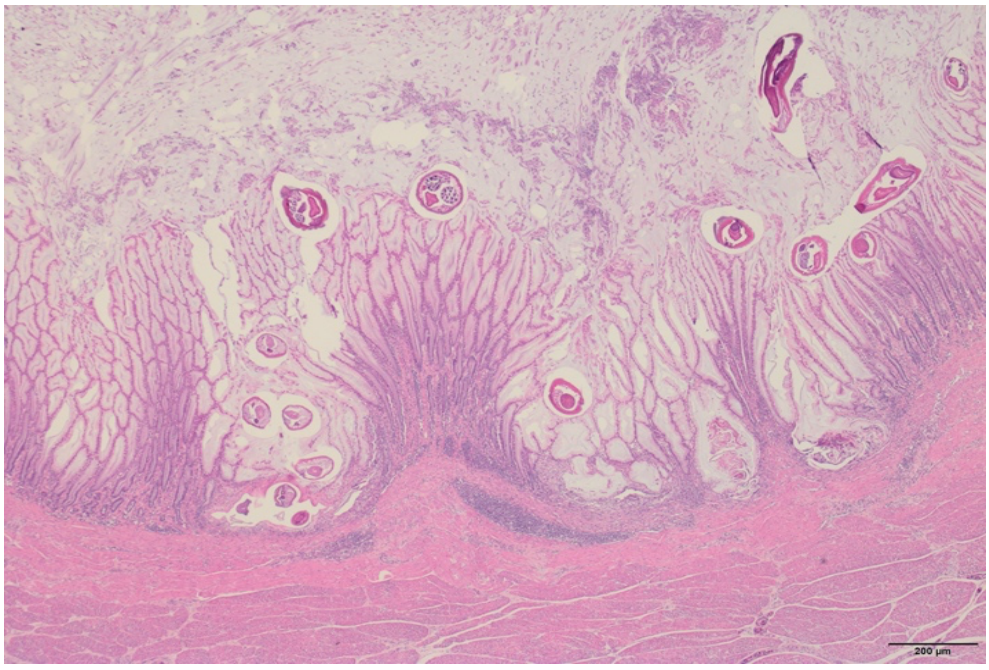

Figure 2.2 Histopathology of affected ventriculus from a brown kiwi (*Apteryx mantelli*) (case 4) showing sections of the ventricular nematodes at the margin of the koilin and mucosa, and deep within the glands of the mucosa.

2.4. Case Discussion

This case series of four kiwi confirm that ventricular nematodiasis can be a significant cause of ill-health and contribute to the mortality of young kiwi. The clinical signs of illness are not pathognomic, and diagnosis depends on gastroscopic examination of the ventriculus in live birds, and histological examination of the ventricular wall in dead birds.

In the early management of the clinically ill kiwi, the first 24 hours afteradmission of the clinical cases focussed on stabilisation of the patients. This is in keeping with prescribed guidelines for kiwi (Morgan, 2008). Stabilisation typically involves administration of fluids orally, subcutaneously or intravenously; providing a quiet, warm, stress-free environment and analgesia. Opioids like butorphanol are usually used initially as non-steroidal anti-inflammatory drugs are reserved for euhydrated patients with good kidney function (Morgan, 2008). Following stabilisation, further diagnostic testing in the form of radiography, haematological and serum biochemical analyses andcoprological examination pointed to high burdens of coccidiosis and ventricular impaction with stones in cases one and two. The former was treated with toltrazuril which is the most widely used, and currently only recommended, anticoccidial drug in kiwi (Doneley, 2006). Trimethoprim- sulphamethoxazole was also used to increase the efficiency of treatment, (Orobets et al.,

2019) as resistance to toltrazuril alone is emerging in managed kiwi populations (Taylor et al., 2019).

Failure of the patients to thrive prompted further diagnosis using gastroscopy. This technique confirmed the VNK in cases one and two. It also revealed severe gastric mucosal damage in the forms of erosion and ulceration. These are in keeping with findings reported in other aves that suffered from ventricular worms (Herman & Wehr, 1954; McLaughlin & McGurk, 1987). In other species of birds, some cases have been severe enough to cause haemorrhagic to necrotizing ventriculitis with damage extending to the muscularis of the ventriculus (Blakeney & Dimmick, 1971). Another important aspect of the pathogenesis is that several clinical signs result from the parasite competing with the host for nutrition and the impaired ability of the host to digest and assimilate nutrients (Nowicki et al., 1995). These further debilitate the host, in turn affecting its ability to thrive, as was manifest in the ante-mortem cases in this study. The debility and gastric haemorrhage induced in one patient (case two) resulted in severe enough anaemia to warrant a blood transfusion.

The ante mortem cases were treated with fenbendazole and moxidectin. While there is no evidence-based information regarding the treatment regimens best suited for VNK, albendazole and moxidectin have been used successfully in treating strongyle infections of the proventriculus and ventriculus of captive ostriches in

Brazil (de Souza et al., 2012).

The ante mortem cases described have several similarities: both were young kiwi whose clinical signs included loss of appetite, coccidiosis, ventricular stones, melaena/haematochezia, regurgitation, and weight loss. They also shared similar lesions in the ventriculus including erythema, erosions to ulcerations and fibrinosis. The cases were also treated similarly but only one of the two patients survived. The inability of the clinicians to arriveat a correct diagnosis early in the progression of the disease is the most limiting factor to successful treatment. In case one, routine diagnostic faecal tests had failed to detect the ventricular nematodes. As a result, the patient continued to deteriorate despite treatment. Gastroscopy was performed to investigate this decline in health and lead to the diagnosis of VNK. Treatment with anthelminthics, specifically fenbendazole and moxidectin, were effective.This was determined through a second gastroscopic examination performed two weeks after treatment was initiated. It was also observed in case two during histopathology of the ventriculus. While worms were still detected, several were found to be in a degenerative stage. These similarities between the cases highlight the importance of diagnosis and treatment of the infection,early in the course of the disease.

The main difference in the two ante mortem cases discussed is that one had a positive patient outcome while the other did not. Despite the same diagnostic tests and treatment regimen applied to

both, the patient in case twosuccumbed. It had lost 25% of its body weight in the month prior to its admission to hospital. The patient was severely hypoproteinaemic and anaemic. This severe haemodynamic compromise and poor body condition likely worsened the condition of the patient and contributed to the negative outcome.

Several similarities are also present between the post-mortem cases. Both were juvenile kiwi confirmed to be cases of VNK through histopathological examination of the ventriculi. Histopathology allowed characterization of the nematodes as mature or immature based on thepresence of eggs within the uteri of adult females. Both gravid females and immature worms were found in all the necropsy cases described. This impliesthat the infections had been patent despite them going undetected in the patients prior to their death by current standard techniques.

The standard coprological technique that is currently used to diagnose other parasitic infections in kiwi is a faecal flotation in 33% zinc sulphate solution (specific gravity of 1.18-1.20). This has been demonstrated to be ineffective at detecting *Cyrnea* sp. eggs (French, 2021). In the light of this technique's failure, another has yet to be developed. The alternate diagnosticoptions currently available are endoscopy and necropsy. Gastroscopic examination is recommended as a diagnostic test for suspected upper-gastrointestinal abnormalities and endoscope-guided foreign body

removal from the ventriculus (Morgan, 2008). While gastroscopy was successful in these ante mortem cases, it is important to note that visualisation of the nematodes was possible only by flushing the ventricular lumen with fluid which floated the nematode bodies and allowed them to be discriminated from the tags of mucosa present. Further, while this method provided a confirmatory diagnosis, it cannot be applied to every case. The major limiting criterion is the size of the patient. The endoscope available could not be passed to the ventriculus of a kiwi weighing less than 800g (Jolly, personal communication,2020). The patients in cases one and two were larger than this minimum bodyweight and therefore gastroscopy could be employed as part of their diagnostics. However, patients in cases three and four in the described serieswere below this threshold and would not have been suitable candidates for gastroscopy had VNK been suspected ante-mortem. This size limitation to gastroscopy with the current equipment available means that it is not a suitable diagnostic choice in many cases. The significance of this is increased considering that all the cases described herein occurred in young kiwi, which do not reach the minimum weight requirement till soon before they are released into the wild. This leaves post-mortem examination as the only technique that can be applied with accuracy to every case, provided the patient has already died. While this is undoubtedly useful from a scientific perspective, it is not useful clinically in ante-mortem cases.

Both ante mortem cases described in this series were treated for VNK with fenbendazole and moxidectin anthelminthics. These were shown to haveefficacy in clearing infection as demonstrated by follow up gastroscopy (case one) and histopathology (case two). These cases provide only anecdotal evidence for treatment and no clinical trials have been performed to establishthe best course of treatment.

A major limitation in dealing with VNK is the paucity of information regarding the subject. Diseases described in kiwi include avian pox, erysipelas, aspergillosis, malaria, coccidiosis, toxoplasmosis, neural and visceral larval migrans as well as traumatic injuries (Alley & Gartrell, 2019)). Of these, coccidiosis is the most important and prevalent (Doneley, 2006; Taylor et al., 2019). However the helminth infections of kiwi have received much less attention, especially those specific to the ventriculus. The nematodeof the kiwi ventriculus is thought to be *Cyrnea apterycis,* a habronematid (Harris, 1975). While there have been no genetic or molecular studies confirming this, recent morphological descriptions do indicate that worms of the *Cyrnea* genus are the likely aetiology of VNK (French, 2021). It is also worth noting that despite these nematodes being found in routine necropsies for years, they had thought to be incidental findings until recently (French, 2021). A recent increase in understanding of this disease and its occurrence has prompted research into establishing an ante-mortem method of

diagnosis.

2.5. Conclusions

This case series examines four cases of VNK in NIB kiwi — both from ante mortem and post-mortem submissions. The ante mortem cases initially presented for a history of inappetence, coccidiosis and ventricular impaction. The patients developed melaena and weight loss. Repeated faecal examinations did not detect the eggs of *Cyrnea* sp. The diagnoses were achieved subsequently through gastroscopy. The post-mortem cases were diagnosed through histological examination of the ventricular wall. While both gastroscopy and necropsy yielded the confirmatory diagnoses, the inability offaecal examination to detect the parasite is alarming. In addition to a lack of an accurate ante-mortem diagnostic technique, very little is known about the parasite. This means that any treatment regimen used has yet to be standardised and there are few reported successes because there are so fewreported cases. This, however, does not imply that there are few cases of VNKbut rather points to the need of developing a suitable ante-mortem diagnosticmethod.

2.6. References

Alley, M. R., & Gartrell, B. D. (2019). Wildlife diseases in New Zealand: recent findings and future challenges. *N Z Vet J, 67*(1), 1-11. doi:10.1080/00480169.2018.1520656

Blakeney, W. C., & Dimmick, R. W. (1971). Gizzard and intestinal helminths of bobwhite quail in Tennessee. *The Journal of Wildlife Management, 35*(3), 4.

Doneley, B. (2006). Management of captive ratities. In G. Harrison & T. Lightfoot (Eds.), *Clinical Avian Medicine* (Vol. 2, pp. 957-989). Palm Beach, Florida, USA: Spix Publishing.

French, A. (2021). *A prospective survey of gastrointestinal nematodes in kiwi (Apteryx spp.).* (PhD Thesis). Massey University, Palmerston North, New Zealand.

Harris. (1975). Two New Nematodes Parasitic in the Kiwi in New Zealand. *Bulletin of the British Museum*, 7.

Herman, C. M., & Wehr, E. E. (1954). The occurrence of gizzard worms in Canada geese. *The Journal of Wildlife Management, 18*(October), 5.

Hunter, S. (2021). Post mortem findings in kiwi suffering from ventricular nematodiasis. In B. Chidambaram (Ed.), (pp. 1).

Jolly, M. (2020). Gastroscopy of kiwi birds. In B. Chidambaram (Ed.), (pp. 1).

McLaughlin, J. D., & McGurk, B. P. (1987). An analysis of gizzard worm infections in fall migrant ducks at Delta, Manitoba, Canada. *Canadian Journal of Zoology, 65*, 8.

Morgan, K. J. (2008). *Kiwi first aid and veterinary care*. Wellington: Department of Conservation.

Nowicki, A., Roby, D. D., & Woolf, A. (1995). Gizzard nematodes of Canada geese wintering in southern Illinois. *Journal of wildlife disease, 31*(3), 7.

Orobets, V., Larionov, S., Kashkovskaya, L., & Korotova, D. (2019). *Modern approach to the problem of bird eimeriosis: Diagnosis and treatment*. Paper presented at the XII interanational scienctific congerence on agricultural machinery industry.

Robertson, H. A. (2013, 2017). North island brown kiwi. *New Zealand Birds Online.*

Robertson, H. A., Baird, K., Dowding, J. E., Elliott, G. P., Hitchmough, R. A., Miskelly, C. M., McArthur, N., O'Donnell, C. F., Sagar, P. M., & Scofield, R. P. (2016). *Conservation status of New Zealand birds*.

Taylor, H. S., Morgan, K. J., Pomroy, W. E., & McInnes, K. (2019). Apparent lack of efficacy of toltrazuril against Eimeria species affecting brown kiwi (Apteryx mantelli) at a captive rearing facility. *N Z Vet J, 67*(2), 101-104. doi:10.1080/00480169.2018.1541425

Yeates, G. W., Zhao, Z. Q., Hitchmough, R. A., & Stringer, I. A. N. (2012). The conservation status of New Zealand Nematoda. *New Zealand Entomologist, 35*(2), 128-130. doi:10.1080/00779962.2012.686317

de Souza, L. P., Lelis, R. T., Granja, I. R., DaMatta, R. A., & Santos Cde, P. (2012). Efficacy of albendazole and moxidectin and resistance to ivermectin against Libyostrongylus douglassii and Libyostrongylus dentatus in ostriches. *Veterinary Parasitology, 189*(2-4), 387-389. doi:10.1016/j.vetpar.2012.04.030

French, A. (2021). *A prospective survey of gastrointestinal nematodes in kiwi (Apteryx spp.).* (PhD Thesis). Massey University, Palmerston North, New Zealand.

3 Development of an Ante-mortem Coprological test forthe Diagnosis of Ventricular Nematodiasis in Kiwi (*Apteryx* spp.)

3.1. Abstract

Ventricular nematodiasis (VNK) in kiwi is suspected to be caused by a *Cyrnea* sp. of nematode. Coprological examination is a routine diagnostic methodology used for the detection of parasitic infections of the gastrointestinal tract (GIT) in both human and veterinary medicine. There are four standard techniques: faecal smears, centrifugal sedimentation, spontaneous flotation and centrifugal flotation. Of these methods, centrifugal flotation is used routinely for the diagnosis of coccidiosis and helminthiases in kiwi (*Apteryx* spp.). However, it has failed to detect *Cyrnea* sp. ova in kiwi faeces for several years. As a result, VNK has remained a poorly understood condition. The recent increase in its reportage has prompted development of ante-mortem faecal tests that can diagnose VNK through coproscopy. Several solutions of varying specific gravities were tested using centrifugal flotation and centrifugal sedimentation to determine the most accurate coprological test. A pilot study using adult female *Cyrnea* sp. worms as a source of eggs, indicated that solutions of high specific gravities, (spg) such as 80% zinc sulphate, (spg 1.385) had higher rates ofdetection of the ova. Based on these findings, several solutions andmethods were tested on kiwi faeces sampled opportunistically from captiveinstitutions across New Zealand. The results of these experiments indicatethat the most sensitive test for the detection of *Cyrnea* sp. ova in kiwi faeces is

centrifugal flotation in 80% zinc sulphate solution, but the most accurate and cost-effective method is centrifugal sedimentation in 33%zinc sulphate solution. From these initial experiments, a viable, preliminary, laboratory test for routine usage has been identified for the diagnosis of VNK.

3.2. Introduction

Coprological examination began in 1878 as a diagnostic tool for humanhookworm infections (Ballweber et al., 2014). The initial technique used faecalsmears, a procedure still in use today. From this evolved faecal sedimentation,gravitational flotation and combined centrifugation-flotation techniques. Whilea history of faecal examination procedures is beyond the scope of this thesis,it is useful to remember that despite its almost 150 years of constant evolutionand refinement, there still remains no one technique or examination solution that can absolutely identify all parasitic ova, oocysts and eggs of domestic, (Thienpont et al., 1986; Zajac et al., 2002) much less non-domestic, animals. The often-poor results of coprological examinations serve as a reminder that a negative result does not necessarily mean that the patient is free of the parasite in question. It only means that it was not detected (Becker et al., 2016). Despite this, coprological examination remains the current best practice for the diagnosis of gastrointestinal parasitism due to its convenience, ease, low cost and fast results.

Another reason for the widespread use of coprological

examination is its ability to be performed by most laboratories in the world. The corollary to this is that most parasitology laboratories have established coprological methods with a few standard test solutions. The use of a different solution forthe detection of only one parasite in only one host genus, i.e., VNK, may be uneconomical, difficult, and simply not worth a laboratory's efforts. Adapting previously standardized test solutions and methods for the detection of the parasite in question would be much more economically feasible and likely to elicit compliance from technical personnel. Therefore, test solutions routinely used in coprological examinations as well as standard coproscopic techniques were used in this experiment to determine a method of ante-mortem faecal testing for VNK.

3.3. Methods of Coprological Examination

There are several methods of coprological examination used to arrive at a parasitological diagnosis. The principles of each method along with their relative advantages and disadvantages have already been discussed in Chapter 1. It is important to keep in mind that a particular method must be chosen based on the goal required. Such goals may include routine health screening, confirmation of a presumptive diagnosis, quantification of parasite burdens or assessing the efficacy of an anthelminthic treatment (Bowman, 2021). This section details the actual techniques and methods involved in eachprocedure. While all different, all the methods require glass examination slides,

coverslips, a light microscope and a fluid medium to either dilute or concentrate the faecal sample.

3.3.1. Slide Preparation and Examination

A sample of the faeces to be examined must be prepared for microscopic evaluation. Regardless of the method used, the sample is typically evaluated by making a smear on a glass slide. To prepare the smear,a small quantity of faeces is mixed thoroughly in water, saline or another appropriate solution (discussed below). While the volume of faeces chosen forthis depends on the particular diagnostic method being employed, a representative sample must be chosen. The volume used ranges from 0.5-2g(Bowman, 2021). The sample in fluid is placed onto a glass slide. The use of coverslips on top of the fluid is encouraged to improve optics and avoid soilingof the microscope (Bowman, 2021). The sample prepared must be thin enough to read through (Thienpont et al., 1986).

The slide is examined under low magnification (10x). Higher magnifications (40x) are usually reserved for morphological identification, measurement and minute parasites like *Giardia* and *Cryptosporidium* spp. (Thienpont et al., 1986). The entire area of the coverslip should be examined from one corner to the other by moving across it in increments of one field- width (Bowman, 2021). Further, any delays in examination can lead to osmoticdistortion of the parasites by the suspension medium or drying out of the coverslip (Bowman, 2021).

For this reason, the slide should be examined as soon as possible after preparation.

3.3.2. Direct Examination

This is the simplest of the coprological methods. A pinhead volume of faecal sample to be examined is placed on a glass side. This sample is dilutedusing a few drops of tap water or normal saline. A coverslip is placed on the sample and the slide is examined under low magnification.

This method can be modified by using a stain such as Lugol's iodine instead of water/saline to dilute the sample. This will impart a yellow colour tomost parasites which makes their identification easier (Thienpont et al., 1986).

3.3.3. Centrifugal and Spontaneous Flotation

For this technique, a standard volume of faecal sample such as 0.5-1 gis weighed. To this a small quantity of flotation fluid is used. The types of flotation fluids available and commonly used are discussed in section 3.4.2. The faeces and fluid are thoroughly mixed and strained through a sieve or mesh. The filtrate is poured into a standard test tube. More flotation medium is added until a slight positive meniscus at the top of the test tube is achieved.A coverslip is placed over the tube such that it is in contact with the fluid (French, 2021).

The test tube is allowed to stand for at least 15 minutes before

the coverslip is examined in the case of spontaneous flotation. Alternately, the process of parasite flotation can be accelerated by centrifuging the test tube. This is the method of centrifugal flotation (Thienpont et al., 1986). The common times and forces used for the centrifugation are discussed in section 3.4.1.

3.3.4. Centrifugal Sedimentation

Centrifugal sedimentation uses the force of a centrifuge to separate theheavier elements including some nematode eggs from the rest of the faecal debris by pushing them to the bottom of the column. This was first used in 1908 (Thienpont et al., 1986) by creating a suspension of faeces in ether andhydrochloric acid. Modern techniques make use of different suspension mediaincluding tap water, ether and ether-formalin (Soares et al., 2020). A fixative such as 10% formalin in water or saline may be included in the procedure priorto mixing with the solvent (Manser et al., 2016). The faecal sample to be examined is measured to a standardized volume, such as 1g, (Manser et al., 2016) mixed in a small volume of solvent, such as ether, and filtered. The mesh size for this varies from 425-1500 μm (Manser et al., 2016). The filtrate is then centrifuged, the speed and time of which are usually calibrated to individual centrifuges such that the parasite elements are forced into the sediment. The sediment so obtained is mixed thoroughly and a drop of it is examined under low magnification (Manser et al., 2016).

3.4. Factors affecting accuracy of coprological methods

The governing parameter for selecting a particular coprological evaluation method is recommended to be simplicity (Thienpont et al., 1986). However, the validity of coprological tests is judged by their sensitivity (ability to avoid false negatives) and accuracy (ability to recover all the parasites in agiven sample) (Becker et al., 2016). The sensitivity of a particular method is calculated as the number of positive test samples divided by the total numberof true positive samples. The accuracy can be calculated in samples where the parasite load is known as the number of parasites recovered divided by the number of parasites present in the sample (Becker et al., 2016). Sensitivity, accuracy and simplicity are influenced by the stage of infection, the techniqueitself and the reagents/solutions utilized (Christie et al., 2011).

3.4.1. Types of Coprological Examination

Although coprological examination is inherently insensitive in detecting parasite burdens, (Christie et al., 2011) its accuracy can be increased by selecting the most appropriate technique for the target parasite. For example, sedimentation methods are more sensitive for trematode and acanthocephalan eggs, amoebae, ciliates and formalin-fixed *Giardia* cysts. However, flotation methods are better at detecting coccidial oocysts, unfixed *Giardia* cysts/trophs or cestode and most nematode eggs (Bowman, 2021). Of the common methods used, benchtop

procedures including direct faecal smears, gravitational flotation and specialized faecal flotation kits (Eg: Ovassay (SqueezeTest, Jorgensen Laboratories Inc.)) produce significantly fewer positive results than procedures that use centrifugation (Dryden et al., 2005; Zajac et al., 2002). Despite being more costly, laborious and elaborate, (Ballweber et al., 2014) sedimentation/flotation methods relying on centrifugation maximisereliability in recovering positive samples (Becker et al., 2016).

Additional technical considerations include proper tube selection, filling,coverslip removal and the type of centrifuge used. The centrifuge must accelerate, run and decelerate smoothly. The time required to run the centrifuge should be calibrated for every individual machine (due to differences in rotor radii and force applied) and preferably described in relativecentrifugal force (g) rather than RPM (Ballweber et al., 2014). Using two coverslips per sample, i.e., analysing a sample twice using two different coverslips consecutively, as well as allowing the tube to stand for 10 minutes after removal from the centrifuge have been shown to increase parasite recovery. The use of special cups/tubes with built-in filters (Ovassay) or removable screens prevent debris from floating up and improve parasite recovery (Ballweber et al., 2014; Dryden et al., 2005).

3.4.2. Types of Flotation Media Used

Although some authors contest that the type of flotation medium used plays no part in technical accuracy, (Dryden et al., 2005), several others speakof its significance (Becker et al., 2016; Christie et al., 2011; Zajac et al., 2002).In some studies, there is a significant influence of the type of solution used onthe outcome of the results (Becker et al., 2016).

While several differing flotation solutions may be used in routine examinations, (Table 3.1) they all function by allowing less dense materials tofloat. In other words, a solution must have a spg higher than the parasite ova/oocysts/eggs being looked for (Ballweber et al., 2014). As most parasiteshave a spg of 1.05-1.23, (Dryden et al., 2005) most flotation solutions have spg of 1.18-1.30 (Ballweber et al., 2014). The eggs of some parasites have a higher sensitivity in a particular solution than others due to their differing spg (Becker et al., 2016). For example, the eggs of *Nanophytes salminicola*, (a trematode) float in a minimum spg 1.275 while solutions of spg 1.20 or less will give a false negative result (Ballweber et al., 2014). Similarly, the eggs of *Procyrnea* sp. (a nematode) float in Sheather's sugar solution of spg 1.27 (Gallo et al., 2018) but are less likely to float in lower density solutions. However, the higher the solution's spg, the higher the osmotic pressure on theparasite ova/eggs and the more severe their distortion. Such solutions will also cause more debris to float which can make parasite identification difficult. Therefore, every solution must strike a balance between having

a spg high enough to float parasites but not so high as to cause their distortion orobstruction by debris (Ballweber et al., 2014).

Table 3.1 Commonly used flotation solutions for coprological investigations (Modified from (Dryden et al., 2005))

Chemical Reagent	Specific Gravity	Concentration	Preparation
Zinc sulphate	1.18-1.20	33% w/v	331g zinc sulphatein 1000ml warm water
Magnesiumsulphate	1.20	45% w/v	450g magnesium sulphate in1000ml water
Sodium nitrate	1.18-1.20	33% w/v	338g sodiumnitrate in 1000ml water
Common salt/Sodium chloride	1.18-1.20	Saturated	Dissolve sodiumchloride in 1000mlwarm water till saturation
Sucrose	1.22	Saturated	Dissolve sucrosein 1000ml warm water till saturation
Modified Sheather's solution	1.27	Saturated	Dissolve 454gsucrose in 355mlwater over a double boiler andadd 6ml formaldehyde

There is also evidence to suggest that the type of reagent used affects the parasites that will float. For example, potassium iodomercurate (spg 1.44)was found to be unable to float *Fasciola hepatica* eggs but floated *Dicrocoelium dendriticum* eggs (Ballweber et al., 2014). Similarly, *Trichuris vulpis* and *Toxocara canis* eggs were found to float significantly more in sugarsolution of 1.2 spg than zinc sulphate of the same spg (Dryden et al., 2005). Further, canine spirurids are best detected using sodium nitrate as the flotationmedium (Christie et al., 2011). It is clear that the method of diagnosis/flotationneeds to take into consideration the parasite in question as some solutions work on specific pathogens while others do not (Ballweber et al., 2014).

Of the commonly used flotation media, 33% zinc sulphate (spg 1.20) isthe standard and preferred reagent used for both sedimentation and centrifugation tests in several parasitological labs. Although its widespread use stems from its ability to float and diagnose human *Giardia* cysts, there are several other factors contributingto its systematic use. It provides a good balance between detection of parasitic ova, oocysts and eggs while minimally distorting all (Becker et al., 2016; Zajac et al., 2002). It is also easily available, non-toxic, low in cost, easy to use and has minimal environmental impact (Ballweber et al., 2014). While these are features common to several flotation solutions, zinc sulphate also has a long shelf-life (Becker et al., 2016) and provides better results for parasites of domestic animals when compared to sugar solution

(Zajac et al., 2002). In fact, it was shown to have 100% sensitivity in a study on some common parasites of domestic species (Becker et al., 2016). It is for these reasons that the standard solution used for coprological examinations is 33% zinc sulphate.

3.5. Methods

3.5.1. Pilot study

Previous laboratory experimentation (French, 2021) revealed that eggsfrom the kiwi ventricular spirurids are poorly detected by standard flotation solutions. As an initial step in developing a more accurate flotation method, a pre-trial was conducted using ethanol-fixed adult female worms and fivedifferent flotation media. This pilot study was performed to provide an initial assessment of various flotation media with respect to their ability to float, and consequently detect, kiwi spirurid eggs.

Solutions of various specific gravities were used as the density of the nematode eggs in question remains unknown. Standard solutions of 50% magnesium sulphate (spg 1.265) and saturated sucrose (spg 1.220) were used. Additionally, as 33% zinc sulphate had proved ineffective at detecting the nematodes, (French, 2021) three different concentrations of zinc sulphatewere used: 60%, (spg 1.300) 68% (spg 1.380) and 80% (spg 1.385). The solutions were prepared freshly and their specific gravities were tested using hydrometers.

Ten adult female ventricular nematodes were identified and

isolated using a dissecting microscope. Their uteri were teased open manually using a blunt probe under 10x magnification and eggs removed using the probe. The eggs, uteri and nematode tissue fragmentswere collected in 15ml test tubes.

Centrifugal flotation was performed by mixing the contents of each testtube with one of solutions to the volume required to produce a slight positive meniscus. A coverslip was placed on top. As ten worms were used, two samples were available for every solution. A fixed-head centrifuge was used at 314g (1200 rpm) for 5 minutes to float the samples. The samples were examined using a compound microscope under 40x magnification byremoving the coverslip onto a glass slide.

3.5.2. Sampling for Experimentation

3.5.2.1. Faecal collection

Samples of kiwi faeces were obtained opportunistically from several conservation centres. Captive kiwi institutions perform routine faecal examinations on juvenile and pre-translocation birds to prevent and detect gastrointestinal helminthiases (Morgan et al., 2012). In addition, several kiwi houses were contacted by the author to request additional samples from routine faecal collection and pen clean ups that would not have ordinarily been sent to SoVS. The institutions that participated in this research project include Pūkaha National Wildlife Centre, Mt Bruce; Kiwis for Kiwis (Westshore); Willowbank Wildlife Reserve, Christchurch; The National Aquarium, Napier and National

Kiwi Hatchery, Rainbow Springs Rotorua. Two wildlife veterinary hospitals, Wildbase Hospital, Massey University and The Nest Te Kōhanga, Wellington Zoo also provided samples from their inpatient kiwi.

All faecal samples were obtained after voiding. Most samples were collected from juvenile kiwi between 3-6 months of age. This is because the wildlife institutions involved in kiwi conservation management usually rear kiwi until they reach 0.8- 1kg in weight (Bassett, 2012).

Once collected, the samples were stored in plastic pottles or zip lock bags and labelled with the date of collection as well as the details of the kiwi from which they were obtained. These details included the name, age and species of kiwi. Sex was not included or considered. The samples were then couriered to the Parasitology laboratory of the SoVS, Massey University. Analysis was performed within 24-72 hours of receiving the samples. The samples were stored at 4^{o}C before and after analysis.

3.5.2.2. Post-Mortem Sampling

Samples were also taken during routine necropsies of kiwi performed through the Massey University Wildbase Pathology Service. Kiwi over two weeks of age were sampled as part of this research project. The ventriculus with its contents and gut contents from the colon to cloaca were collected. Theventriculi were stored in 70% ethanol to aid

in worm fixation (Graves & Fedynich, 2013). Analysis was performed within 24-72 hours of receiving the samples. The samples were stored at 4^{o}C before and after analysis.

3.5.2.3. Time period of Sampling

Sampling was conducted for nine months from November 2020 to July2021. This corresponds to one season of kiwi rearing from hatch to release through the ONE project. A seasonal fluctuation in sample numbers was observed (Figure 3.1) with the maximum number of samples occurring in the autumn months and the fewest samples coming during the winter months. Thiswas observed to be true for both faecal and post-mortem samples.

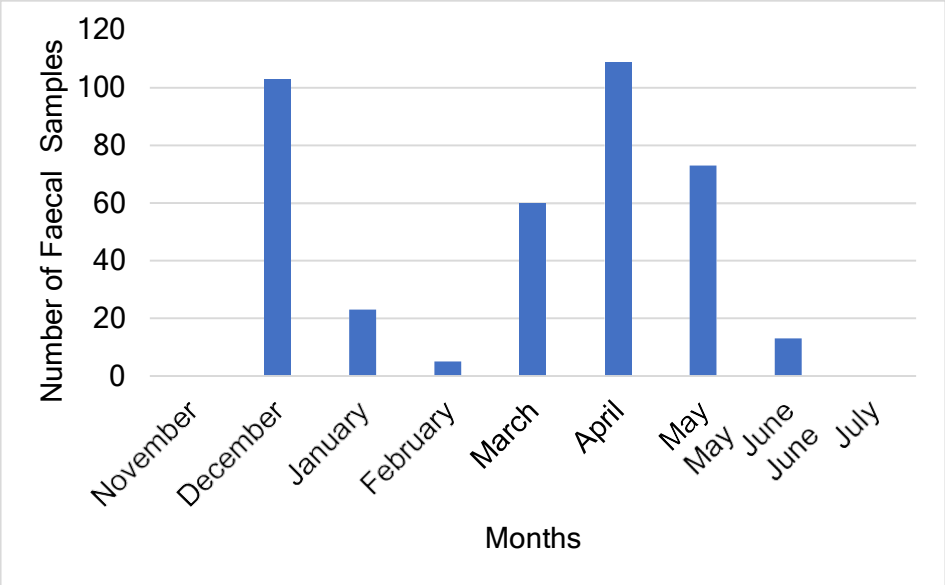

Figure 3.1 Time period of sampling and number of samples for the determination of *Cyrnea* spp. ova in kiwi faecal samples

3.5.3. Examination of Samples

3.5.3.1. Faecal samples

All faecal samples were analysed by centrifugal faecal flotation. The samples were mixed thoroughly and 0.5g was weighed out. This sub-sample was mixed with 15ml of 80% zinc sulphate flotation solution (spg 1.385) and filtered to remove debris. The filtrate was transferred to a standard 15ml test tube such that a slight positive meniscus was achieved and a 2x2cm^2 coverslipwas placed over the test tube so it was in contact with the filtrate within the tube. The test tube and coverslip, so prepared, were placed in a fixed-head type centrifuge and spun for 5 minutes at 314g (1200 rpm). The sample was removed and the coverslip was transferred to a plain glass slide. The slide wasthen examined under x100 magnification using a compound microscope (Olympus CX-31, Olympus Life Science Solutions). All nematode eggs of the spirurid parasite under investigation were counted. These were identified based on their morphological characteristics of asymmetrical shape, presence of a single opercula and yellow-brown colour. As 0.5g of sample was used, the result obtained was multiplied by two (x2) to obtain the parasite burden as eggs per gram (EPG) of faeces. Samples containing one or more eggs of the nematode understudy were considered to be positive for VNK. These results are presented in Appendix 1 at the end of this thesis (page 121)

All positive samples were tested with a variety of faecal flotation

solutions. This was done in an attempt to calculate the relative sensitivity of each solution and identify the test that provided the highest number of parasites per sample. However, owing to the limited volume of samples obtained in several cases, not all of the solutions could be tested on every sample. As a result, solutions which had shown consistently negative results such as saturated sugar were tested after other solutions, provided enough sample volume was present. s

Positive samples were tested using the flotation solutions mentioned intable 2. The method of faecal flotation remained unchanged from the procedure described above. In April 2021, another flotation solution was added to the experiment — 33% sodium nitrate (spg 1.18). This solution was included in the test protocol due to the poor results obtained from a few other solutions and as it had shown good results with canine spirurid eggs (Christieet al., 2011).

3.5.3.2. Centrifugal Sedimentation of PositiveSamples

A subset of samples (n = 16) determined to be positive by faecal flotation in 80% zinc sulphate were also tested by centrifugal sedimentation. Every sample was mixed thoroughly and 0.5g was weighed. The weighed sample was then mixed with a low spg solution, in this case, 33% zinc sulphate (spg 1.18) and filtered. The filtrate was transferred to a standard 15ml test tubeand the volume was made up to the 15ml mark with more test solution, if needed. The test tube so prepared was centrifuged for 5 minutes in a fixed- head centrifuge at

314g (1200 rpm). The supernatant was then removed using a 1ml Samco pipette attached to a 20 ml syringe. This allowed a controlled volume to be removed from the test tube. 12.5 ml out of 15ml was suctioned from the test tube, leaving the pellet and a small volume of supernatant such that the total volume left was 2.5ml. This test fluid was thoroughly mixed and 0.25ml was transferred onto a glass slide using a dropper. The sample on theslide was covered with a 2x2cm^2 coverslip and examined at x100magnification using a compound microscope (Olympus CX-31). As only 0.25ml of a 2.5ml pellet sample was examined, the number of eggs identified in the coverslip sample was one-tenth of that present in the total test fluid volume. Further, as only 0.5g of faeces was used, a multiplication factor of twenty (x20) was applied to every count performed under the microscope for the centrifugal sedimentation.

3.5.3.3. Post-Mortem Samples

The intestinal contents from dead kiwi were tested by faecal flotation in80% zinc sulphate, as described, to detect the nematode eggs while the ventriculi and their contents were examined for the occurrence of the whole worms. The presence of worms in the ventriculi was intended to serve as a positive control for the faecal flotation method employed to detect the larvae. Worms were identified by both gross examination and histopathology of the ventriculus.

3.5.4. Statistical Analysis

Correlation analysis along with linear regression was performed to evaluate the different diagnostic methods. The methods were tested relative to flotation in 80% zinc sulphate solution as this method was employed as thebenchmark to diagnose incoming samples as positive or negative based on results of the pilot study. Pearson correlation and simple linear regression were employed on flotation in 80%, 68% and 60% zinc sulphate and 50% magnesium sulphate, and sedimentation in 33% zinc sulphate.

The Bland-Altmann (B-A) plot is a method of evaluating the differences between two quantitative measurements (Morgan & Aban, 2016). The B-A plotis a graphical plot whose abscissa is the mean of the two tests and the ordinate is the difference between them. The data points are restricted within twice the standard deviation(s) from the mean of the tests which serve as limits of agreement (LOA) and interpreted against the mean difference in test results (bias) (Giavarina, 2015; Morgan & Aban, 2016). Whether these LOA are acceptable or not must be defined *a priori* (Giavarina, 2015). The B-A plot allows comparison of the tests by quantifying the differences between their ability to measure a common variable. This contrasts with evaluating the correlation between two tests which only highlights the similarities between them. For this reason, correlation analysis alone is not recommended to assess the comparability between two methods (Giavarina, 2015).

The B-A plot method was used to assess the differences between those tests that had the highest detection rates of EPG. For these plots, the level ofsignificance of bias and LOAs were decided *a priori.* As the purpose of these tests were to detect the presence of eggs from a potentially pathogenic nematode, which have remained undetected for several years, even small differences in bias and LOA are important.

3.6. Results

3.6.1. Pilot Study

The pilot study revealed that the test flotation solution which provided the highest eggs per worm count was 80% zinc sulphate solution (mean ± se: 173 ± 33). This solution had the highest spg of those tested (spg 1.385). The egg per worm count was found to reduce with the spg of the solutionsused. The results are presented in Table 3.2.

It is worth noting even though the number of eggs present in the uterus of every female adult worm varies, this number is assumed to be the same in the pilot study to allow for easy comparison of the results

Table 3.2 Results of a pilot study for detection of *Cyrnea* sp. ova in kiwi faecal samples

Solution	Specific gravity	Eggs per slide		
		Test 1	Test 2	Average
s80% Zinc sulphate	1.385	140	206	173
68% Zinc sulphate	1.380	60	45	52.5
60% Zinc sulphate	1.300	3	0	1.5
50% Magnesium sulphate	1.265	0	0	0
Saturated Sucrose	1.22	0	0	0

3.6.2. Faecal Sampling

Using 80% zinc sulphate as the initial screening test, 38 out of 450 kiwifaecal samples (8.4% test prevalence) were identified as positive for the *Cyrnea* sp. eggs, the causative agent of VNK.

Comparison of the faecal flotation solutions was then carried out on the positive faecal samples. Overall, it was observed that 33% sodium nitrate (0/18; 0% sensitivity) and 33% zinc sulphate (0/36; 0% sensitivity) solutions did not detect any nematode eggs. Saturated sugar (1/14; 7.1% sensitivity) and 50% magnesium sulphate solutions (11/32; 34.3% sensitivity) were also relatively insensitive compared to 60% (10/33; 30.3% sensitivity) and 68% (17/34; 50% sensitivity) zinc sulphate solutions. Of the faecal flotation methods, 80% zinc sulphate, which had been used to isolate the positive samples initially, proved to detect the greatest number of nematode eggs as indicated by the linear

regression, B-A plots (Figures 3.3 and 3.2) and correlation analysis (Table 3.3).

On examination of the BA plots, when 68% flotation and 80% flotation solutions are compared, the bias is -26.12 and no discernible trend can be observed from the plot with most data points lying close to the bias line. Though there is a wide difference between the lower and upper LOA, the lack of trend and narrow bias indicate that these tests are in general agreement (Table 3.4).

Table 3.3 The matrix of correlation analyses comparing the diagnostic methods tested for the detection of *Cyrnea* sp. ova in kiwi faecal samples

		80% $ZnSo_4$	68% $ZnSO_4$	Centrifugal sedimentation	60% $ZnSO_4$	50% $MgSO_4$
80% $ZnSO_4$	Pearson Correlation	1	0.602	0.745	0.496	0.196
	Sig. (2-tailed)		<0.001	0.001	0.003	0.283
	N	36	34	16	33	32
68% $ZnSO_4$	Pearson Correlation	0.602	1	0.455	0.832	0.387[1]
	Sig. (2-tailed)	<0.001		0.076	<0.001	0.035
	N	34	34	16	33	30
Centrifugal sedimentation	Pearson Correlation	0.745	0.455	1	0.328	.[2]
	Sig. (2-tailed)	0.001	0.076		0.215	<0.001
	N	16	16	16	16	15
60% $ZnSO_4$	Pearson Correlation	0.496	0.832	0.328	1	0.602
	Sig. (2-tailed)	0.003	<0.001	0.215		<0.001
	N	33	33	16	33	30
50% $MgSO_4$	Pearson Correlation	0.196	0.387[1]	.[2]	0.602	1
	Sig. (2-tailed)	0.283	0.035	<0.001	<0.001	
	N	32	30	15	30	32

[1] Significant at 0.05 level

[2] Was not computed because at least one of the variables is constant

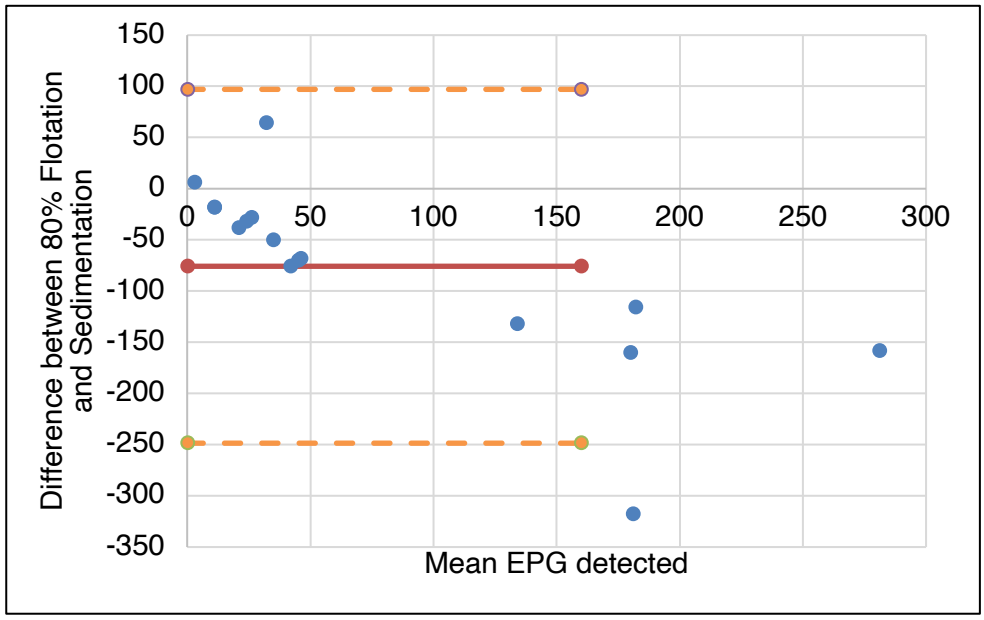

Figure 3.2a B-A Plot of 80% Flotation vs Sedimentation

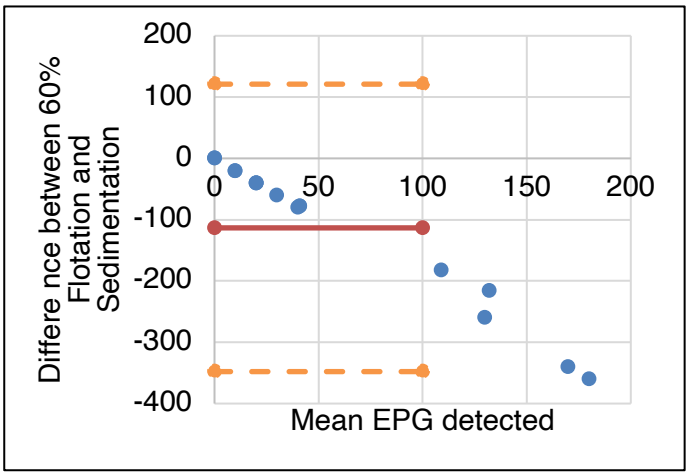

Figure 3.2b. B-A plot of 60% Flotation vs. Sedimentation

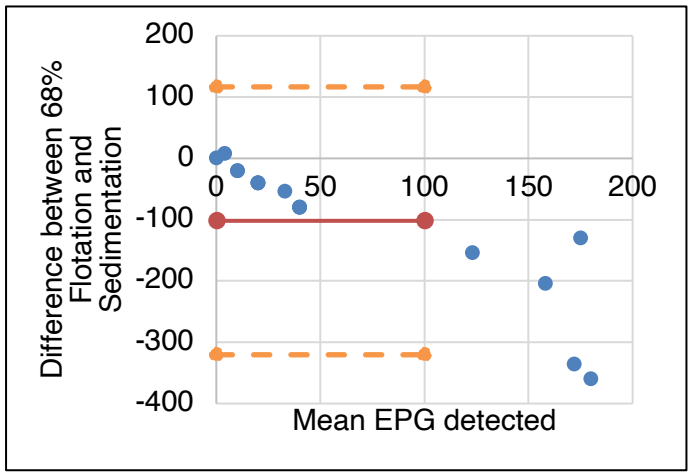

Figure 3.2c. B-A plot of 68% Flotation vs. Sedimentation

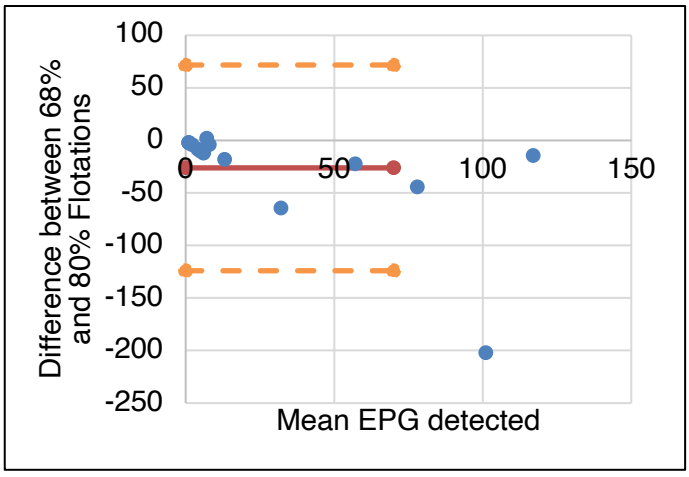

Figure 3.2d B-A plot of 60% vs. 80% Flotations

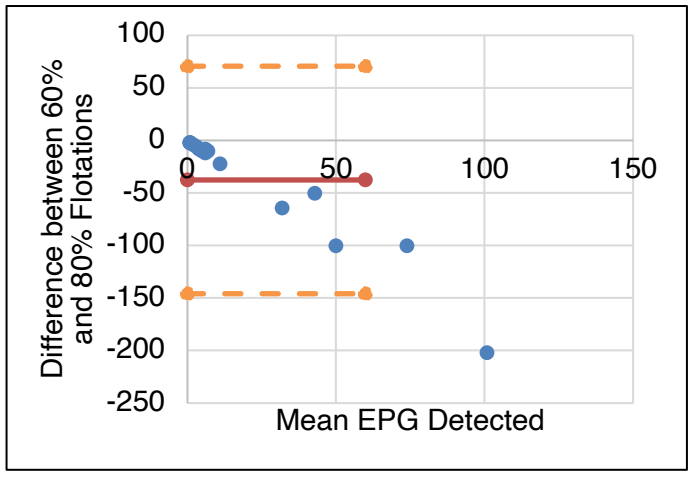

Figure 3.2e B-A plot of 68% vs. 80% Flotations

Figure 3.2 Bland-Altman plots comparing the diagnostic methods tested for the detection of *Cyrnea* sp. ova in kiwi faecal samples.

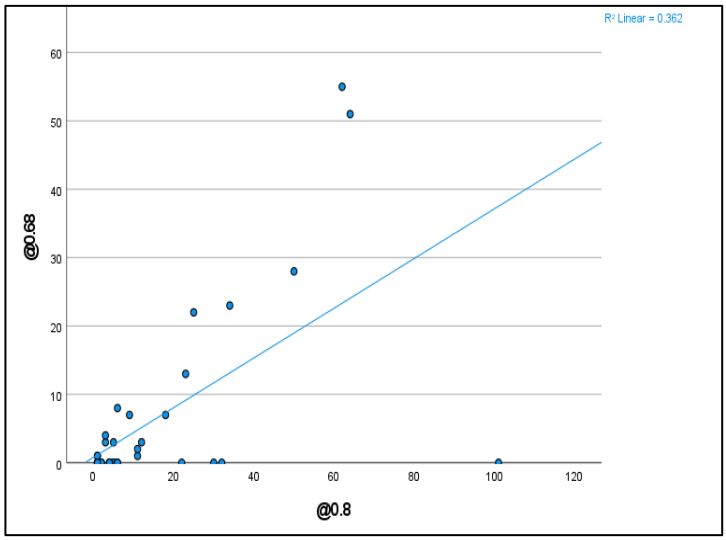

Figure 3.3a Scatter plot and linear regression of 80% and 68% Flotations

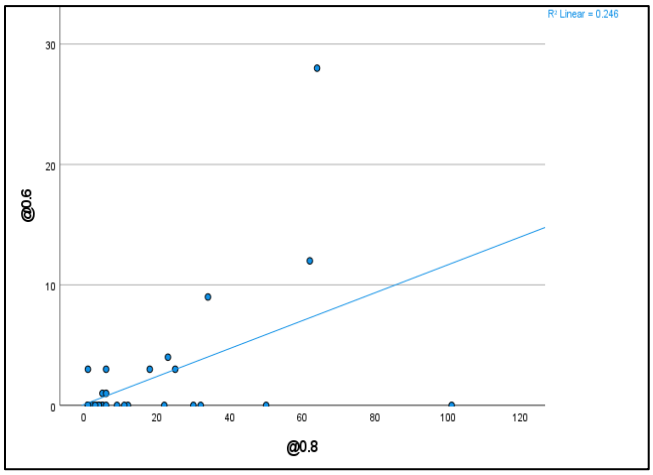

Figure 3.3b Scatter plot and linear regression of 80% and 60% Flotations

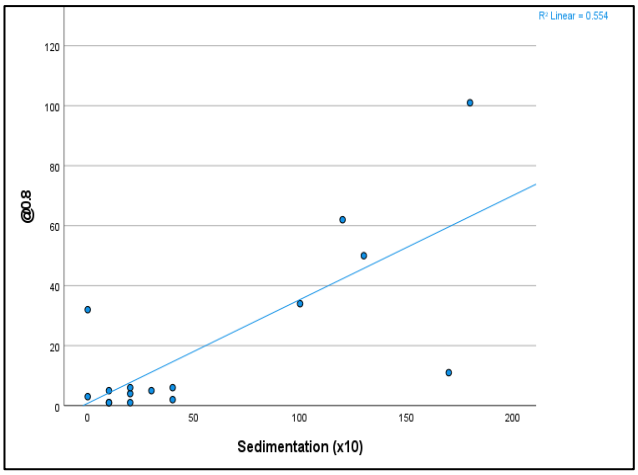

Figure 3.3c Scatter plot and linear regression of 80% Flotation and Sedimentation

Figure 3.3 Scatter plot and linear regression comparing the detection of *Cyrnea* sp. ova in kiwi faecal samples between flotation and sedimentation tests show that a poor predictability of the tests when compared to each other

Linear regression was performed to compare the detection of *Cyrnea* sp. ova in kiwi faecal samples between 80% and 68% zinc sulphate flotation tests and between the 80% and 60% zinc sulphate flotation tests. The regression showed the tests had poor predictability (R^2 = 0.362; Figure 3.3a and R^2 = 0.246; Figure 3.3b respectively).

Table 3.4 Summary of Bland-Altman Plots comparing the diagnostic methods tested for the detection of *Cyrnea* sp. ova in kiwi faecal samples

Comparison Tests	Bias	Difference between LOAs	Trend
80% Flotation vs Sedimentation	-75.75	345.59	Negative
68% Flotation vs Sedimentation	-101.87	437.04	Negative
60% Flotation vs Sedimentation	-113.37	468.95	Negative
68% vs 80% Flotations	-26.12	195.79	None discernible
60% vs 80% Flotations	-36.62	216.53	Negative

3.6.3. Centrifugal Sedimentation of PositiveSamples

Centrifugal sedimentation performed on a subset of 16 positive samples showed higher sensitivity (14/16; 87.5% sensitivity) than other flotation methods apart from that in 80% zinc sulphate. Further, the quantitative EPG count (obtained after applying the multiplication factor) was higher (mean ± s.e. = 112.5±30.98) than previously obtained from the faecal flotation in all solutions including the 80% zinc sulphate (mean ± s.e. = 31.61±7.44). Otherwise, the centrifugal sedimentation method showed a highcorrelation (r=0.745; p = 0.001) to the 80% zinc sulphate flotation results (Table 3.3, Figure 3.2a).

B-A plot analysis of 80% flotation vs sedimentation revealed that the bias was -75.75 and the LOA ranged from -248.55 to 97.04. These along withthe negative trend indicate that the tests are not in agreement.

Linear regression was performed to compare the detection of *Cyrnea* sp. ova in kiwi faecal samples between 80% zinc sulphate flotation and centrifugal sedimentation. These tests had the closest degree of association ($R^2 = 0.554$) (Figure 3.3a).

3.6.4. Post-mortem sampling

Over the time-period of sampling, at post-mortem, five kiwi were juveniles (<6months) from captive institutions and were considered in the experimental sampling. Of these kiwi, 4 out of 5 had confirmed VNK based on evaluation of the ventriculi (Table 3.5). This evaluation involved careful examination of the ventricular contents as well as mucosa as many adult nematodes were found buried in the ventricular wall. In addition, histopathology of the ventriculus was performed to aid diagnosis. Unfortunately, 60% of the samples obtained through necropsy were not suitable for faecal examination. The specimens often did not have any material present in the intestines after the level of the colon, making examination of their faeces impossible. Only two of the kiwi examined had sufficient colonic-cloacal content for coprological examination and both were negative on faecal flotation using 80% zinc sulphate solution.

Table 3.5 Results of post-mortem sampling and faecal testing for *Cyrnea* sp. ova in kiwi

Kiwi	Adult worms in ventriculus	Faecal sample	Test in 80% Flotation
A	Present	Present	Negative
B	Present	Present	Negative
C	Present	Absent	NA
D	Present	Absent	NA
E	Absent	Absent	NA

3.6.5. Costs of Analyses

The relative costs required to prepare all the experimental solutions is detailed in Table 3.6 (prices current at time of writing). While the most cost- effective solutions are those of sodium nitrate, magnesium sulphate and sugar, they have very poor sensitivity and provided minimal EPG counts per sample. The difference between 33% and 80% zinc sulphate is $9.40/100ml in their costs of preparation. However, the increased cost of using zinc sulphate over other reagents is overcome by the higher detection rates and ease of use it provides.

As these differences in cost result from the solutions used, the only differences between faecal sedimentation and flotation as methods of analysis would depend the examination solution used.

Table 3.6 Relative costs of faecal examination solutions used for the detection of *Cyrnea* sp. ova in kiwi faecal samples

Chemical Reagent	Solution (%)	Cost/100ml ($ NZD)	Cost/test ($ NZD)
Zinc sulphate	80	16.00	1.06
	68	13.60	0.91
	60	12.00	0.80
	33	6.60	0.44
Sodium nitrate	33	3.96	0.26
Magnesium sulphate	50	0.15	0.01
Sugar	Saturated	0.15	0.01

3.7. Discussion

Several methods of coprological examination were tested and compared in order to determine the most appropriate method of ante-mortem diagnosis of VNK. Of the methods employed, seven were centrifugal faecal flotations and one was a centrifugal sedimentation technique. They all used 0.5g homogenised kiwi faeces that had been collected opportunistically from captive institutions across New Zealand.

All the samples were initially tested by centrifugal flotation in 80% zinc sulphate solution based on the findings of the initial pilot study. Those found positive were re-tested by all the other methods: flotations in 33%, 60%, 68%zinc sulphate; 33% sodium nitrate; 50% magnesium sulphate; and saturated sugar solution. A subset of the positive samples was later tested usingcentrifugal sedimentation in 33% zinc sulphate. As

there was no gold-standardtest or positive control that could have served as the test against which all others could be compared, the experimental tests were compared to each other to determine the most suitable amongst them. This was performed through correlation analysis, linear regression and B-A plot analysis to determine the relative sensitivity of the methods and which test provided the highest EPG count for each sample.

The correlation analysis and linear regression compared the tests employed against flotation in 80% zinc sulphate solution as this was the platform test used to distinguish a sample as positive or negative. The correlation coefficients and coefficients of determination obtained were used to determine the degree to which the tests were in agreement with each other.Based on the results, the strongest association was found to be between flotation in 80% zinc sulphate solution and sedimentation in 33% zinc sulphatesolution. It is unsurprising that these two tests have the highest agreement as they both had the highest rates of EPG detection in the test samples. Further,the correlation between 80% zinc sulphate flotation and tests that had a very poor rate of EPG detection, such as 50% magnesium sulphate flotation, werevery low.

In order to better compare the tests, B-A analysis was performed. Thisis a statistical test recommended for comparison of diagnostic tests because it evaluates the differences between them rather than the similarities (as correlation analysis does) (Giavarina, 2015). This test is also useful because its results are interpreted from a clinical

perspective. This analysis revealed that there were significant differences between most of the tests employed. The practical implication is that one test should not substitute the other in a clinical setting.

On examination of the B-A plot of 80% flotation compared to sedimentation (Figure 3.2a) a bias of -36 was found. This is interpreted to mean that for every sample tested by both methods, sedimentation would detect on average 36 EPG more than flotation.

The LOA between the two tests ranged from -128.83 to 51.89. All but one of the data points fell within this range. Clinically speaking this means thatthere is a wide difference in the ability of the tests to detect the nematode eggsin the samples. This difference is potentially 180.72 EPG with a 95% CI. It indicates a poor agreement between the tests. This is also demonstrated by the negative trend in the B-A plots where the bias reduces the higher the meanEPG count gets (Table 3.4).

Sedimentation in 33% zinc sulphate solution had an average detectionrate of 36 EPG more than 80% zinc sulphate flotation, but a slightly lower sensitivity. As the ability to detect even one EPG is considered clinically important for this experiment, the smallest quantifiable difference between various tests is the most important criterion for their selection for widespread use. The B-A analysis showed that sedimentation in 33% zinc sulphate also had clinically significant higher mean EPG detection rates than 68% zinc sulphate flotation and 60% zinc sulphate flotation. The same was found to betrue of 80% zinc

sulphate flotation when compared to 60% zinc sulphate flotation. These results imply that the test which provides the best quantification of *Cyrnea* sp. eggs in a given sample of kiwi faeces is centrifugal sedimentation in 33% zinc sulphate solution.

Centrifugal sedimentation has additional pragmatic advantages. The test solution is of much lower concentration than most others used in this experiment, i.e., 33% vs 50-80%. This means that less volume of chemical is required to make the same volume of test solution which translates to a lowercost per ml of test solution, making the overall cost of the test lower. The costsof preparation of 100ml of various solutions are detailed in Table 3.6. In addition, 33% zinc sulphate is routinely used in parasitology laboratories for centrifugal and spontaneous flotation tests (Dryden et al., 2005). It is also usedfor the examination of kiwi faecal samples at the Massey University Parasitology Laboratory for detection of coccidiosis through centrifugal flotation. This means that while the supernatants from the processed samples can be checked for the presence of coccidia and nematode species such as *Heterakis* sp., the sediment of the same samples can be checked for *Cyrnea* sp; i.e., the two different tests can be performed on the same sample that needs to be processed and centrifuged only once. This would require minimum sample volume and reduce the time spent in sample processing as well as cutting the costs required. Centrifugal sedimentation using 33% zinc sulphate solution is thus the most accurate test currently available for the detection of *Cyrnea* sp. eggs in kiwi faeces while also being easy,

cheap and quick.

3.8. Limitations

The major limitation of this study was the lack of an existing standardised test to compare the tested methodologies against. The use of post-mortem sampling was attempted to instead provide a positive control as the presence of adult nematodes in the ventriculus would indicate a patent infection. The coprological technique that would best detect eggs in the colonic-cloacal contents of such specimens would have served as the method against which others could have been compared. However, only a few such samples were obtained and even fewer samples were suitable for faecal testing. The reason this might be is uncertain but is likely related to the debilitated condition of most necropsy specimens – most of these kiwi had suffered from illnesses prior to death which likely caused anorexia and consequently inhibited faecal output. To overcome this limitation of limited faecal samples from post-mortem investigations, the coprological methods tested were compared to each other.

Another limitation is that not all the methods were tested on all the samples. The use of sodium nitrate solution and sedimentation were developed during the course of this investigation and could not be applied to the samples collected at the start of the experiment. While the

test results from sodium nitrate flotation were insignificant, the sedimentation method proved toyield the highest EPG counts. Although this test is less sensitive than the 80%flotation, its ability to detect more EPG per sample indicates its higher accuracy in determining pathogen load. Whether it can accurately detect positive samples that 80% zinc sulphate flotation cannot is undetermined in this study. It remains an area for future research and is required to better establish the test of choice for detection of *Cyrnea* sp. ova in kiwi faecal samples.

The use of coprological testing is inherently limiting because it can never detect infections caused by immature nematodes. For eggs to be present in the faeces of a patient, it must have adult worms within its system as only adults can lay eggs. If the infection is a result of immature worms, regardless of the coproscopic technique employed, the infection cannot be detected. In such cases diagnosis is usually the result of gastroscopic or post-mortem investigation. This means that coprological testing can never provide 100% sensitivity in diagnosing VNK, or any parasitic infection.

3.9. Conclusion

An ante-mortem test to diagnose VNK was formulated and refined using coprological testing. The results of this study indicate that the most sensitive method to detect the nematode eggs is 80% zinc sulphate flotation but that centrifugal sedimentation in 33% zinc sulphate solution is more effective in quantifying the number of ova present. Centrifugal

sedimentation has the additional benefits of being cheap, easy to use and quick. Further, kiwifaecal samples that are routinely tested for coccidia and other parasites usingfaecal flotation can be examined for VNK in the sediment from the same sample. This reduces the time and effort required for sample processing, making it the economic choice. Although further testing is required to categorically determine the test of choice for diagnosis of VNK, a viable preliminary test for laboratory use has been identified from this study.

3.10. References

Alley, M. R., & Gartrell, B. D. (2019). Wildlife diseases in New Zealand: recentfindings and future challenges. *N Z Vet J, 67*(1), 1-11. doi:10.1080/00480169.2018.1520656

Avelar Ide, O., Almeida, L. R., Santos, H. A., Lima Wdos, S., Lara, L. B., & Ecco, R. (2014). Sicarius uncinipenis and Deletrocephalus cesarpintoi in captive greater rheas of Minas Gerais State, Brazil. *Rev Bras Parasitol Vet, 23*(3), 355-359. doi:10.1590/s1984-29612014053

Bagnato, E., Frixione, M., Digiani, M. C., & Cremonte, F. (2018). A new species of Procyrnea (Nematoda: Habronematidae) parasitic in Rhea pennata (Aves: Rheidae) from Patagonia, Argentina, with a key to species of the genus. *J Helminthol, 92*(4), 504-513. doi:10.1017/S0022149X17000657

Ballweber, L. R., Beugnet, F., Marchiondo, A. A., & Payne, P. A. (2014). American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use--is there really one best technique? *Vet Parasitol, 204*(1-2), 73-80. doi:10.1016/j.vetpar.2014.05.009

Banda, M. E., Howe, L., Gartrell, B. D., McInnes, K., Hunter, S., & French, N. P. (2013). A cluster of avian malaria cases in a kiwi management programme. *NZ Vet J, 61*(3), 121-126. doi:10.1080/00480169.2012.736130

Barlow, S. (2018). *Captive management plan for kiwi*. Australia: Zoo and Aquarium Association 2018

Bassett, S. (2012). *Operation nest egg incubation and chick rearing best practice protocols*. Wellington: Oralis Limited

Becker, A. C., Kraemer, A., Epe, C., & Strube, C. (2016). Sensitivity and efficiency of selected coproscopical methods-sedimentation, combined zinc sulfate sedimentation-flotation, and McMaster method. *Parasitol Res, 115*(7), 2581-2587. doi:10.1007/s00436-016-5003-8

Blakeney, W. C., & Dimmick, R. W. (1971). Gizzard and intestinal helminths of bobwhite quail in Tennessee. *The Journal of Wildlife Management, 35*(3), 4.

Borgsteede, F. (2005). The gizzard worm, Amidostomum acutum (Lundahl, 1848) Seurat, 1918 in common eiders (Somateria mollissima L.) in the Netherlands. *Helminthologia, 42*, 4.

Bowman, D. D. (2021). Helminths. In *Georgis' parasitology for veterinarians* (11 ed., pp. 135-260). USA: Elsevier.

Choe, S., Kim, H., Lim, J., Lee, D., Park, H., Jeon, H.-K., Kim, H., Kim, Y., & Eom, K. S.(2016). New Record of Schistorophus cirripedesmi (Nematoda: Acuariidae)from a Bar-Tailed Godwit, Limosa lapponica baueri (Charadriformes: Scolopacidae) in Korea. *The Korean Journal of Parasitology, 54*(3), 349-355. doi:10.3347/kjp.2016.54.3.349

Christie, J., Schwan, E. V., Bodenstein, L. L., Sommerville, J. E. M., & van der Merwe, L. L. (2011). The sensitivity of direct faecal examination, direct faecal flotation, modified centrifugal faecal flotation and centrifugal sedimentation/flotation in the diagnosis of canine spirocercosis. *Joiurnal of the South African Veterinary Association, 82*, 6.

Clark, W. C. (1978). Procyrnea keasp. nov. (Habronematidae: Spirurida: Nematoda) from the New Zealand Kea (Nestor notabilisGould, 1865) (Aves: Psittaciformes). *Journal of the Royal Society of New Zealand, 8*(3), 323-328.

doi:10.1080/03036758.1978.10429384
David, E. D., & Lindquist, W. D. (1982). Determination of the Specific Gravity of Certain Helminth Eggs Using Sucrose Density Gradient Centrifugation. *The Journal of Parasitology, 68*(October 1982), 4.
Deplazes, P., Eckert, J., Mathis, A., von Samson-Himmelstjerna, G., & Zahner, H. (2016). Order Spirurida. In *Parasitology in veterinary medicine* (1 ed., pp.346-356). The Netherlands: Wageningen Academic Publishers.
Doneley, B. (2006). Management of captive ratities. In G. Harrison & T. Lightfoot(Eds.), *Clinical Avian Medicine* (Vol. 2, pp. 957-989). Palm Beach, Florida, USA: Spix Publishing.
Dryden, M. W., Payne, P. A., Ridley, R., & Smith, V. (2005). Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts.
Veterinary Therapeutics, 6(Spring), 14.
Dunn, A. M. (1978). Superfamily Spiruridoea. In *Veterinary helminthology* (2 ed.,Vol. 1, pp. 69-72). Glasgow: Butler & Tanner ltd.
Ebmer, D., Wiedermann, S., & Sattmann, H. (2020). Morphological identifications of gastrointestinal nematodes and acanthocephalans of raptors and owls from Austria. *Annalen des Naturhistorischen Musems in Wien, B, 122*, 6.
Ederli, N. B., & de Oliveira, F. C. R. (2014). Macroscopic lesions of the ventriculus of Rhea americana , Linnaeus, 1758 (Aves: Rheidae) naturally infected by Sicarius uncinipenis (Molin, 1860) (Nematoda: Habronematidae). *J Parasitol,100*(6), 860-863. doi:10.1645/13-416.1
Ederli, N. B., & de Oliveira, F. C. R. (2019). Redescription of Procyrnea uncinipenis (Molin, 1860) (Nematoda: Habronematidae) based on material from Rhea americana (L.) (Aves: Rheidae). *Syst Parasitol, 96*(8), 735-745. doi:10.1007/s11230-019-09880-w
Ederli, N. B., Gallo, S. S. M., & de Oliveira, F. C. R. (2020). Natural infection by Procyrnea uncinipenis (Nematoda, Habronematidae), a parasite from rheas, an autoctone bird from South America, in emus Dromaius novaehollandiae, a ratite from New Zealand. *Sci Rep, 10*(1), 16541. doi:10.1038/s41598-020- 73754-1
Ferrell, S. T., Pope, K. A., Gardiner, C., Bradway, D. S., Ambrose, D. L., MacLean, R. A., Norton, T. M., Stedman, N. L., & Garner, M. M. (2009). Proventricular nematodiasis in wrinkled hornbills (Aceros corrugatus). *Journal of Zoo and Wildlife Medicine, 40*, 8.
French, A. (2021). *A prospective survey of gastrointestinal nematodes in kiwi (Apteryx spp.).* (PhD Thesis). Massey University, Palmerston North, New Zealand.
Friend, M., & Franson, J. C. (1999). Gizzard worms. In E. A. Ciganovich (Ed.), *Field manual of wildlife diseases feneral field procedures and diseases of birds* (pp. 438): U.S. Department of the interior U.S. Geological survery.
Gallo, S. S. M., Ederli, N. B., & Oliveira, F. C. R. (2018). Endoparasites and ectoparasites of rheas (Rhea americana) from South America. *Tropical Biomedicine, 35*, 12.
Ganapathy, K., & Sharma, R. S. K. (2003). Cheilospirura species (gizzard worm) infection in battery-caged commercial layer chickens. *Veterinary Record,153*, 2.
Germano, J., Barlow, S., Castro, I., Colbourne, R., Cox, M., Gilles, C., Hackwell, K., Harawira, J., Impey, M., Reuben, A., Robertson, H. A., Scrimgeour, J., Sporle, W., & Yong, S. (2018). *Kiwi Recovery Plan 2018-2028*. (64). Wellington: Department of Conservation

Giavarina, D. (2015). Understanding Bland Altman analysis. *Biochem Med (Zagreb), 25*(2), 141-151. doi:10.11613/BM.2015.015

Graves, D. W., & Fedynich, A. M. (2013). Assessing Helminth Community Structure and Patterns in Gizzard Helminths of Blue-Winged Teal (Anas Discors). *Journal of Parasitology, 99*(5), 748-751. doi:10.1645/ge-3240.1

Harris. (1975). Two New Nematodes Parasitic in the Kiwi in New Zealand. *Bulletin of the British Museum*, 7.

Herman, C. M., & Wehr, E. E. (1954). The occurrence of gizzard worms in Canada geese. *The Journal of Wildlife Management, 18*(October), 5.

Lymbery, A. J., Morine, M., Kanani, H. G., Beatty, S. J., & Morgan, D. L. (2014). Co-invaders: The effects of alien parasites on native hosts. *Int J Parasitol Parasites Wildl, 3*(2), 171-177. doi:10.1016/j.ijppaw.2014.04.002

Manser, M. M., Saez, A. C., & Chiodini, P. L. (2016). Faecal Parasitology: Concentration Methodology Needs to be Better Standardised. *PLoS Negl Trop Dis, 10*(4), e0004579. doi:10.1371/journal.pntd.0004579

McLaughlin, J. D., & McGurk, B. P. (1987). An analysis of gizzard worm infections in fall migrant ducks at Delta, Manitoba, Canada. *Canadian Journal of Zoology, 65*, 8.

Mejia-Fava, J., Divers, S. J., Jimenez, D. A., Ambrose, D. L., Rech, R., Gottdenker, N. L., & Mayer, J. (2013). Diagnosis and treatment of proventricular nematodiases in an umbrella cockatoo. *Journal of American Veterinary Medical Association, 242*, 5.

Moller, A. P. (1997). Parasitism and the evolution of host life history. In D. H. Clayton & J. Moore (Eds.), *Host-parasite evolution: General principles and avian models* (pp. 105-127). Oxford: Oxforn University Press.

Moller, A. P., & Erritozoe, J. (1996). Parasite virulence and host immune defense: host immune response is related to nest reuse in birdds. *Evolution, 50*(5), 7.

Morgan, C. J., & Aban, I. (2016). Methods for evaluating the agreement between diagnostic tests. *J Nucl Cardiol, 23*(3), 511-513. doi:10.1007/s12350-015- 0175-7

Morgan, K. J. (2008). *Kiwi first aid and veterinary care*. Wellington: Department of Conservation.

Morgan, K. J. (2013). *Coccidiosis in the kiwi (Apteryx spp.) : aspects of the pathology, epidemiology and parasite biology : a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Veterinary Science at Massey University, Palmerston North, New Zealand.* (Doctor of Philosopy (Ph.D.). Doctoral). Massey University, Retrieved from http://hdl.handle.net/10179/6340

Morgan, K. J., Alley, M. R., Pomroy, W. E., Castro, I., & Howe, L. (2012). Enteric coccidiosis in the brown kiwi (Apteryx mantelli). *Parasitol Res, 111*(4), 1689-1699. doi:10.1007/s00436-012-3008-5

Naem, S., Houston, R. S., & Sentíes-Cué, G. (2012). New insights into morphological features of Hadjelia truncata (Spirurida: Habronematidae), as revealed by SEM. *Parasitology Research, 112*(1), 327-333. doi:10.1007/s00436-012-3140-2

Niemuth, J. N., Allgood, J. V., Flowers, J. R., De Voe, R. S., & Troan, B. V. (2013). Ventricular Habronemiasis in Aviary Passerines. *Case Reports in Veterinary Medicine, 2013*, 1-6. doi:10.1155/2013/719465

Norton, D. A. (2009). Species invasions and the limits to restoration: Learning from the New Zealand experience. *Science, 325*(July), 4.

Nowicki, A., Roby, D. D., & Woolf, A. (1995). Gizzard nematodes of Canada geese wintering in southern Illinois. *Journal of wildlife disease, 31*(3), 7.

Orobets, V., Larionov, S., Kashkovskaya, L., & Korotova, D. (2019). *Modern approach to the problem of bird eimeriosis: Diagnosis and treatment*. Paper presentedat the XII interanational scienctific congerence on agricultural machinery industry.

Predator Free Trust. (2020). Predator free 2050 vision. *The big picture.* Retrievedfrom https://pf2050.co.nz/the-predator-free-movement/

Razmi, G. R., Kalidari, G. A., & Maleki, M. (2007). First report of the Hadjelia truncata infestation in pigeons of Iran. *Iranian journal of veterinary research,8*(19), 4.

Robertson, H. A. (2013, 2017). North island brown kiwi. *New Zealand Birds Online.*

Robertson, H. A., Baird, K., Dowding, J. E., Elliott, G. P., Hitchmough, R. A., Miskelly, C. M., McArthur, N., O'Donnell, C. F., Sagar, P. M., & Scofield, R. P. (2016). *Conservation status of New Zealand birds*.

Smith, B. L., Poole, W. S. H., & Martinovich, D. (1973). Pneumoconiosis in thecaptive new zealand kiwi. *Veterinary Pathology, 10*, 8.

Soares, F. A., Benitez, A. D. N., Santos, B. M. D., Loiola, S. H. N., Rosa, S. L., Nagata, W. B., Inacio, S. V., Suzuki, C. T. N., Bresciani, K. D. S., Falcao, A. X., & Gomes, J. F. (2020). A historical review of the techniques of recovery of parasites fortheir detection in human stools. *Rev Soc Bras Med Trop, 53*, e20190535. doi:10.1590/0037-8682-0535-2019

Taylor, H. S., Morgan, K. J., Pomroy, W. E., & McInnes, K. (2019). Apparent lack of efficacy of toltrazuril against Eimeria species affecting brown kiwi (Apteryxmantelli) at a captive rearing facility. *N Z Vet J, 67*(2), 101-104. doi:10.1080/00480169.2018.1541425

Taylor, M. A., Coop, R. L., & Wall, R. L. (2016). Veterinary parasitology, 4th Edi. In: Wiley Blackwell, UK.

Thienpont, D., Rochette, F., & Vanparijs, O. F. J. (1986). *Diagnosing helminthiasis by coprological examination* (Vol. 1986): Janssen Research Foundation Beerse, Belgium.

van Zyl, N. (2014). *Nematodiasis and larval migrans in kiwi (Apteryx spp.) : a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Zoology at Massey University, Palmerston North, New Zealand.*(Master of Science (M.Sc.) Masters). Massey University, Retrieved from http://hdl.handle.net/10179/6322

White, D. J., Hall, R. J., Wang, J., Moore, N. E., Park, D., McInnes, K., Gartrell, B. D., &Tompkins, D. M. (2016). Discovery and complete genome sequence of a novel circovirus-like virus in the endangered rowi kiwi, Apteryx rowi. *Virus Genes, 52*(5), 727-731. doi:10.1007/s11262-016-1342-x

Yeates, G. W., Zhao, Z. Q., Hitchmough, R. A., & Stringer, I. A. N. (2012). The conservation status of New Zealand Nematoda. *New Zealand Entomologist,35*(2), 128-130. doi:10.1080/00779962.2012.686317

Zajac, A. M., Johnson, J., & King, S. E. (2002). Evaluation and importance of centrifugation in zinc sulphate flotations. *Journal of the American AnimalHospital Association, 38*, 4.

Zettermann, C. D., Nascimento, A. A., Tebaldi, J. A., & Szabo, M. J. (2005). Observations on helminth infections of free-living and captive rheas (Rheaamericana) in Brazil. *Vet Parasitol, 129*(1-2), 169-172. doi:10.1016/j.vetpar.2004.12.015

Zhang, L., Brooks, D. R., & Causey, D. (2004). Procyrnea Chabaud 1958 (Nematoda:

habrnonemtoidea: habronematidae) in birds form the area de conservacion guanacaste, Costa Rica, including desciptions of 3 new species. *Journal of Parasitology, 90*, 10.

Zhang, S., Song, J., & Zhang, L. (2011). Three species ofProcyrneaChabaud, 1958 (Nematoda: Habronematoidea: Habronematidae) from raptors in Beijing, China, with descriptions of two new species. *Journal of Natural History, 45*(47-48), 2915-2928. doi:10.1080/00222933.2011.622451

4 The Prevalence of *Cyrnea* sp. Eggs in Faecal Samples from Captive Kiwi

4.1. Abstract

The prevalence of parasitic diseases in kiwi remains poorly understood, with ventricular nematodiasis in kiwi (VNK) being no exception. Two initial studies on gastro-intestinal parasitism in kiwi indicated that nematodes may be present in up to 88% of kiwi ventriculi. However, both these studies utilizedpost-mortem samples. A study was therefore designed to determine theprevalence of VNK in kiwi using ante-mortem faecal sampling. The samples were tested using centrifugal flotation in 80% zinc sulphate solution. Sampleshaving at least one egg per gram of faeces were determined to be positive for infection. The samples were obtained opportunistically from kiwi in nine captive institutions located throughout New Zealand. This study estimated thatthe prevalence of *Cyrnea* sp. ova in kiwi faeces from captive institutions was 16.77% (95% CI) as determined through coprological investigations. This study also examined the effects of host species, age and location on the disease prevalence. While no significant relationship between host location and infection prevalence could be determined, this study revealed that North Island Brown kiwi (*Apteryx mantelli*) and kiwi less than 6 months in age were the most likely to be affected. However, these estimates were the result of opportunistic sampling in only captive kiwi. The prevalence in wild populationsof kiwi remains unknown and studies on the environmental transmission of the

parasite are needed to determine the effect, if any, of location on disease prevalence.

4.2. Introduction

Very few parasites have been identified from kiwi (*Apteryx* spp.). Even fewer have been isolated from the ventriculus. Recent studies indicate, however, that the parasites present in the ventriculus of kiwi are responsible for causing clinical illness. The pathogen responsible for this VNK is believed to belong to the genus *Cyrnea* in the habronematid family of spirurid nematodes (French, 2021). Unfortunately, little else is known about this disease and the causative agent.

The paucity of information regarding the parasitic diseases of kiwi, especially those specific to the ventriculus, has been well established. The most-well studied parasitic disease of kiwi is coccidiosis. However, there have been only two previous studies regarding the helminthic diseases of kiwi. While one was conducted retrospectively (van Zyl, 2014) and the other prospectively, (French, 2021) they both utilized post-mortem sampling. The results of both these studies varied greatly. The initial retrospective study based on a review of post-mortem records found that the prevalence of VNK detected was 26.3% (van Zyl, 2014). However, the later prospective study, which carried out a much more targeted examination of dead kiwi, found that this was a gross underestimation. It concluded that 94% of kiwi suffered from some degree of gastrointestinal nematodiasis with 88% being

affected in the ventriculus (French, 2021).

Another noteworthy result from both these studies is that they highlight the lack of prevalence study in ante-mortem kiwi samples. The obvious reasonbehind this is that there was no reliable test that could be used to positively detect the parasites in ante-mortem, i.e., faecal, samples. As an ante-mortem test for detection of the parasite in question (*Cyrnea* sp.) has been identified, this is no longer a restriction to such a study. Further, an ante-mortem study would bypass the limitation of relying on gross necropsy and histological findings for confirmation. A necropsy-based diagnosis of ventricular nematodiasis is inapplicable to live hosts. Histological evidence of ventricular nematodes was found in only 57.5% of all the confirmed cases identified in the prospective study (French, 2021). It is therefore a relatively inaccurate method of nematode identification. Ante-mortem faecal testing and disease identification subverts these pitfalls and has all the advantages of coprological diagnosis discussed in chapter three: small sample volume; minimal equipment, time, effort and cost.

The aim of this study was to carry out a prospective survey of faecal samples from captive live kiwi to determine the prevalence of VNK and determine the effects of kiwi species, age and sampling location on test prevalence.

4.3. Materials and Methods

4.3.1. Sampling

Samples of kiwi faeces were obtained opportunistically from several conservation centres. Captive kiwi institutions perform routine faecal examinations on juvenile and pre-translocation birds to prevent and detect gastrointestinal helminthiases (Morgan et al., 2012). In addition, several kiwi houses were contacted by the author to request additional samples from routine faecal collection and pen clean ups that would not have ordinarily beensent for testing. The institutions that participated in this research project include Pūkaha National Wildlife Centre, Mt Bruce; Kiwis for Kiwis, Westshore and The Burrow; Willowbank Wildlife Reserve, Christchurch; The National Aquarium, Napier and National Kiwi Hatchery, Rainbow Springs Rotorua. Two wildlife veterinary hospitals, Wildbase Hospital, Massey University and The Nest Te Kōhanga, Wellington Zoo also provided samples from their inpatient kiwi.

All faecal samples were obtained after voiding. Multiple samples were obtained from some kiwi over the course of the sampling period while some others provided only one sample for this study. Most samples were collected from juvenile kiwi between 3-6 months of age, with no sex bias.

Once collected, the samples were stored in labelled plastic pottles orzip lock bags. The samples were then submitted for analysis. Analysis

was performed within 24-72 hours of receiving the samples. The samples were stored at 4^{o}C before and after analysis. In total, 450 samples were obtainedover an eight-month period from December 2020 to July 2021.

4.3.2. Examination of Samples

All faecal samples were analysed by centrifugal faecal flotation. The samples were mixed thoroughly and 0.5g was weighed out. This sub-sample was mixed with 15ml of 80% zinc sulphate flotation solution (spg 1.385) and filtered to remove debris. The filtrate was transferred to a standard 15ml test tube such that a slight positive meniscus was achieved and a 2x2cm^2 coverslipwas placed over the test tube so it was in contact with the filtrate within the tube. The test tube and coverslip, so prepared, were placed in a fixed-head type centrifuge and spun for 5 minutes at 314g (1200 rpm). The sample was removed and the coverslip was transferred to a plain glass slide. The slide was then examined under x100 magnification using a compound microscope(Olympus CX-31). All nematode eggs of the spirurid parasite under investigation were counted. The result so obtained (eggs present in 0.5g of faeces) was multiplied by a factor of two (x2). This quantified the parasite burden as eggs per gram (EPG) of faeces. Samples containing one or more eggs of the nematode under study were considered to be positive for VNK.

4.4. Statistical analysis

450 samples of kiwi faeces were obtained from captive institutions across New Zealand. The samples were categorized on the following bases: host age, host species, captive institution of origin and presence of *Cyrnea* sp.eggs (positive or negative). In positive cases, the EPG count of the nematodes was also assessed. The names of the institutions that participated in this studyhave been anonymised in the analysis. A total of nine institutions (A to I) participated in this study. The host ages ranged from 6 weeks to 50 years. Theages were further split into five categories for analysis (<3 months, 3-4 months,6-12 months, 1-5 years and + 5 years). There were three species of kiwi identified in this study: North Island brown (NIB) (*Apteryx mantelli*), rowi (*A. rowi)* and great spotted kiwi (*A. haastii).* Due to the large number of Eastern brown kiwi (EB) (*A. australis mantelli)* identified in this study, this genotype was examined separately from other NIB genotypes.

The 95% confidence intervals were calculated by the Wilson method using an online calculator (Epitools; https://epitools.ausvet.com.au/ciproportion). Descriptive statistics, cross-tabulations and ANOVAs for the effect of kiwi species and age on the testprevalence were carried out using SPSS v27 (IBM).

4.5. Results

Of the 450 faecal samples tested, 38 samples were determined to be positive (having at least one EPG) and 412 samples were negative giving a test prevalence (± 95% CI) of 0.084 (± 0.062 - 0.114) (Appendix 1). However, many of the kiwi that were used in this study provided multiple faecal samples. In this study, 167 kiwi were included out of which 28 tested positive for at least one sample. This gives the disease prevalence (± 95% CI) as 0.1677 (± 0.1186 - 0.2317).

4.5.1. Effects of Host Age

There was strong evidence of a significant effect of age on parasite prevalence ($\chi^2 = 13.98$, df = 4, p = 0.007). The age of the kiwi was available for 413 out of the total 450 samples. There were 32/36 (88.9%) positive samples from the 3–4-month age range and 4/36 (11.1%) were seen in kiwi less than three months of age. In other words, the test prevalence was 0.129 in the 3–4-month age and 0.035 in the birds that were less than 3-months of age (Figure 4.1).

There was no evidence (F = 0.137, df = 1,36, p = 0.713) for a differencein the quantitative EPG results from the positive samples from the 3-4 month age range (mean EPG count ± s.e. = 31.56 ± 7.68) and from the birds less than 3 months of age (mean EPG count ± s.e. = 24.67 ± 19.89) (Figure 4.2).

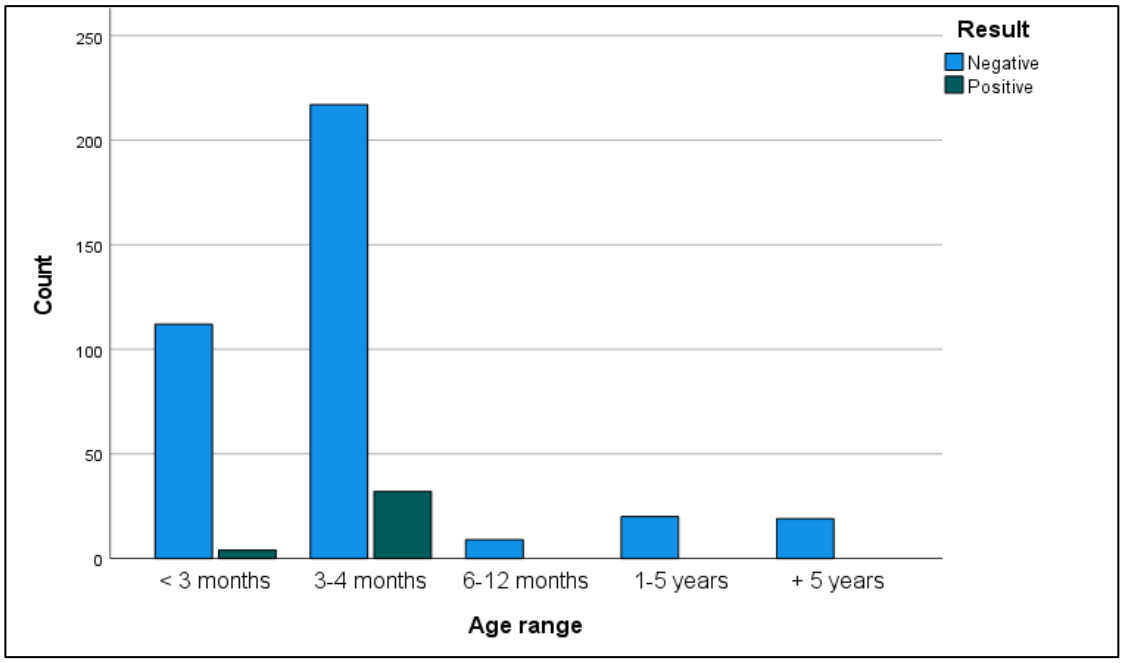

Figure 4.1 Age distribution of faecal samples positive for *Cyrnea* sp. ova from kiwi that kiwi under 4 months of age are the most frequently affected

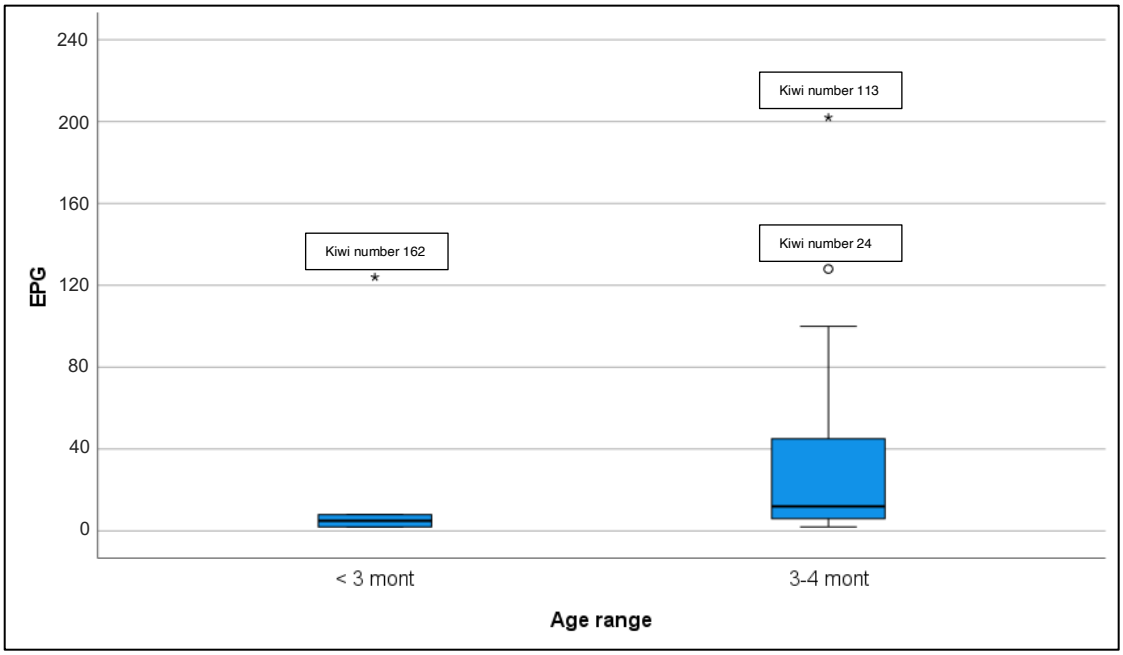

Figure 4.2 The difference in quantitative counts for faecal *Cyrnea* sp. ova between kiwi in the less than 3 months and 3-4 month age group show that the latter suffer higher EPG burdens

4.5.2. Effects of Host Species

The species or genotype of kiwi was able to be identified in 413 out ofthe 450 samples. There was strong evidence ($\chi^2 = 12.685$, df = 3, p = 0.005) for a difference in prevalence between the kiwi species and genotypes (Figure4.3). The positive samples were only found in NIB kiwi hosts. Of this species,the EB kiwi genotype had a test prevalence of 0.023 (3/128) while the other NIB kiwi genotypes had a test prevalence of 0.141 (33/234) (Figure 4.3).

There was no evidence (F = 1.398, df = 1,36, p = 0.245) of a differencein the quantitative EPG counts between the EB genotype (Mean ± s.e. = 2.67

± 0.67) and other genotypes of NIB kiwi (Mean ± s.e. = 32.86 ± 7.57) (Figure 4.4).

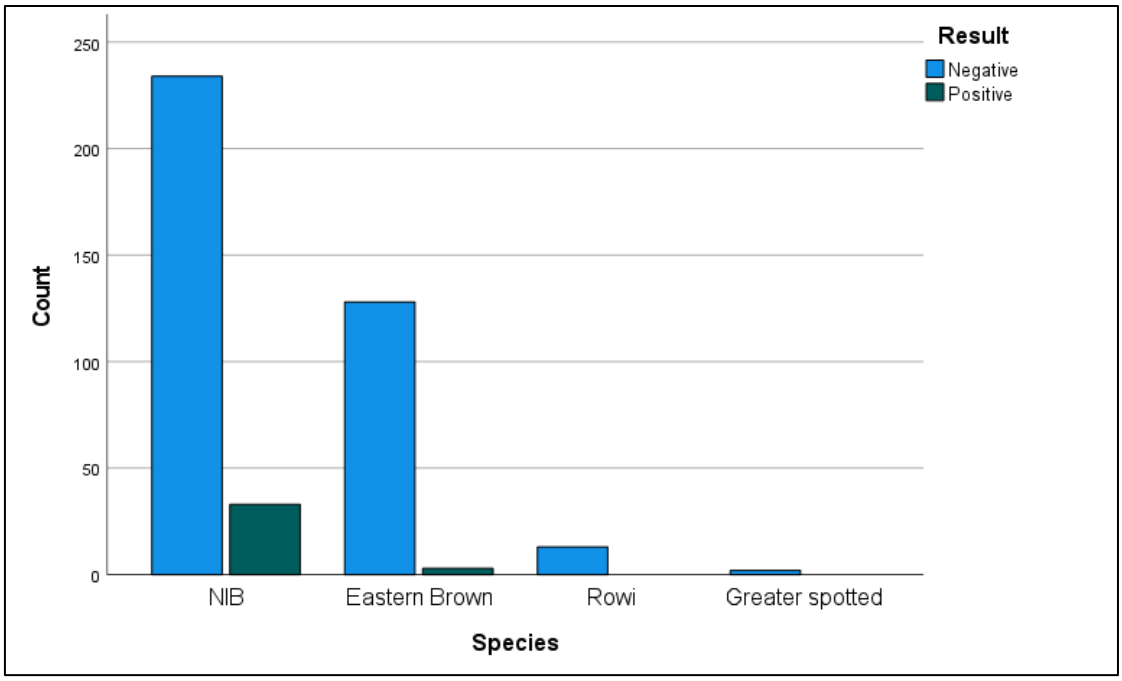

Figure 4.3 Species distribution of faecal samples positive for *Cyrnea* sp. ova from captive kiwi

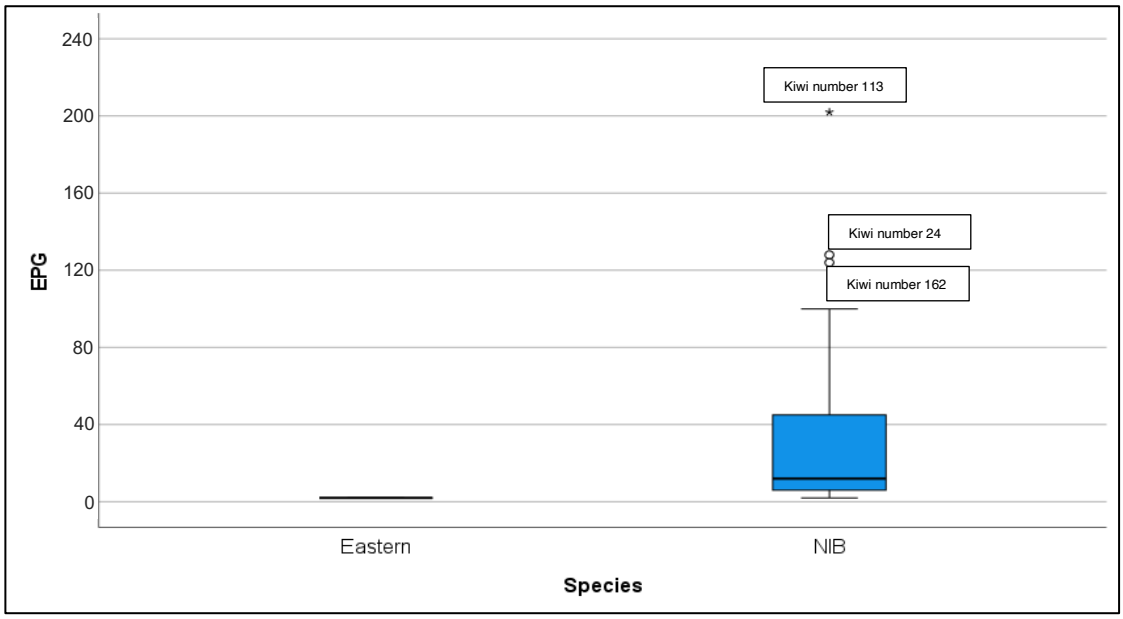

Figure 4.4 The difference in quantitative counts for faecal *Cyrnea* sp. ova between NIB kiwi from the eastern genotype and all other genotypes

4.5.3. Effects of Submission Institution Location

The samples were submitted by nine different institutions across New Zealand. Of these, only one is located in the South Island while the rest are spread over the North Island. The samples were examined with respect to these institutions to determine if these locations had any bearing on the disease prevalence. 413 samples from seven of the locations were analysed.The others were excluded due to insufficient information regarding the submitter or sample itself.

Samples tested positive and negative from several locations as can beseen in Figure 4.5.

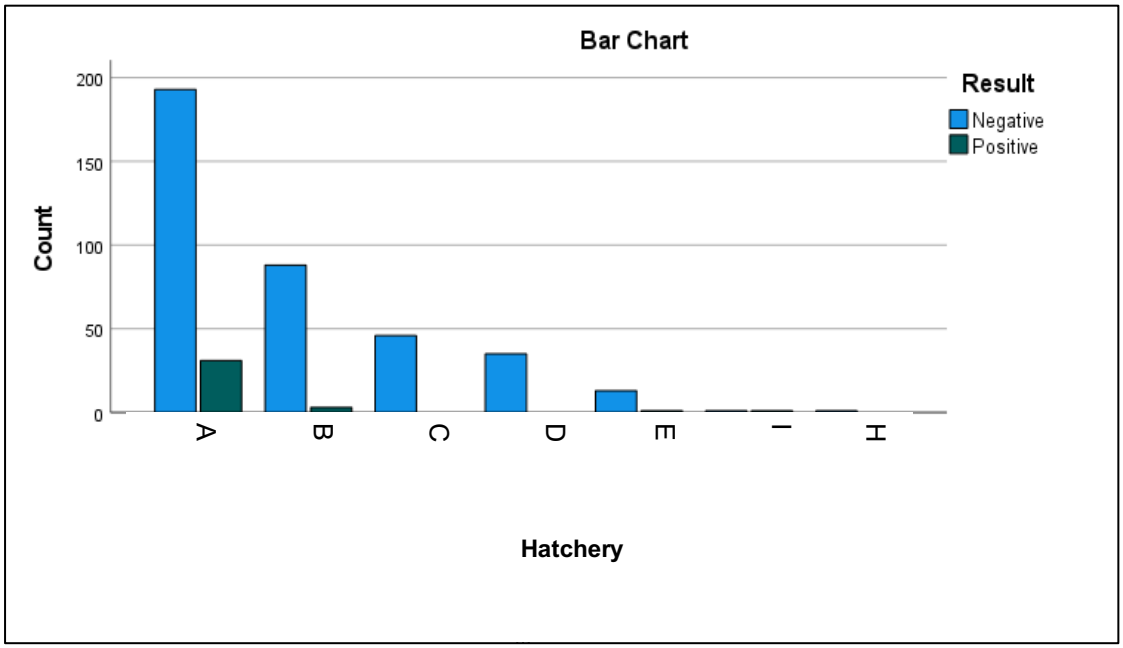

Figure 4.5 Submission location distribution of faecal samples positive for *Cyrnea* sp. ova from kiwi

4.6. Discussion

This study relied on coprological testing to determine prevalence of VNK in captive kiwi. The samples were examined using centrifugal flotation in 80% zinc sulphate solution to determine those that were positive for the eggs of *Cyrnea* sp. Although the centrifugal sedimentation in 33% zinc sulphate solution was found to provide a higher EPG count per sample, (refer chapter 3) this test was developed after the sampling period had concluded and as such was not available at the time of this prevalence study. However, given the correlation between the sedimentation test and 80% flotation test, the results of this prevalence study can still be considered valid. Further, the moresensitive of these tests was shown to be the 80% flotation. Therefore, it was chosen as the method of testing to determine the prevalence.

The distribution of positive and negative samples was analysed with reference to host species, age and submission institution location. Positive samples were identified from only two host age-ranges: < 3 months and 3-4months. Although samples had been received from kiwi as old as 50 years,none of the samples from kiwi older than 4 months were positive. Thisindicates that kiwi 4 months or younger are the most likely to be infected. Thishypothesis was further tested by Pearson's Chi-square test. A statistically significant relationship between host age and infection prevalence was found. Similar tests were performed to determine if there was a relationship between the species of host and

disease prevalence. Positive samples were identified from NIB and EB while samples from rowi and great spotted kiwi were all negative. A Pearson's Chi-square test also determined therelationship between host species and disease prevalence to be statistically significant. These findings indicate that the most likely species of kiwi to be affected by this disease is the NIB, regardless of genotype.

Positive and negative samples were identified from most submission locations. However, as some institutions had only a few samples in this study and others had hundreds, their comparison is difficult. As such, whether submission location has a statistically significant effect on disease prevalence cannot be accurately determined.

The box-plot analyses on the effects of host age and species on EPG counts showed similar findings. Among the positive cases identified, those kiwi younger than 4 months are likely to have EPG counts less than 50. The same can be said for positive cases in NIB and EB kiwi.

The prevalence of VNK captive kiwi based on ante-mortem coprological testing was found to be 16.77%. In comparison, the prevalence of kiwi coccidiosis estimated through similar coprological analysis was found to be 23.80% (Morgan et al., 2014). This disease, although much better understood, is considered the most important gastrointestinal parasitic disease of kiwi (Doneley, 2006) and has been confirmed as the primary cause of mortality in 26% of affected cases (Morgan et al., 2014). Though the prevalence of VNK is less than that of

coccidiosis, reports of it resulting in kiwi mortality and significant morbidity have increased in recent years (refer chapter 2). This may warrant regular faecal screening and prophylactic treatment in susceptible kiwi, similar to coccidiosis. Further, as this is the first study on the ante-mortemprevalence of VNK in captive kiwi, further studies will be required to determineif this prevalence level holds true in wild populations and the pathogenic effectsof the disease.

The ante-mortem prevalence was found to be much lower when compared to the post-mortem prevalence of 88%. The reasons for this come down to the methods of sampling, analyses and the ability of post-mortem investigations to detect infections caused by very low burdens, or by only immature worms which would not shed eggs in the faeces. The necropsy examinations were also performed on kiwi that had died due to a variety of different causes, including other infections, predation and traumatic injuries. Itis reasonable to believe that the immunity of at least some of these specimenswould have been compromised, making them potentially more susceptible to nematodiasis. The ante-mortem sampling was performed on faeces that had been submitted for routine parasitic screening and as such were mostly from otherwise healthy individuals. Accurate comparison of the two prevalence estimates would thus be difficult.

This study evaluated the prevalence in only captive kiwi. Such kiwi are housed together in creches or outdoor pens and are exposed to the same environmental stimuli and pathogens on a daily basis. They

are also providedthe same food and soil substrate. As a result, there is high potential for cross infection and re-infection among them which would weigh heavily on the disease prevalence. The same is thought to be true for coccidiosis in creche- reared kiwi (Morgan et al., 2014; Taylor et al., 2019). Such conditions are not encountered as frequently in wild or free-range populations. The prevalence of disease in wild kiwi is therefore likely to be lower than that estimated in thisstudy.

The prevalence of cases in young birds may speak to an age-resistancein kiwi against this infection. This is thought to occur due to the improved immunity of adults compared to juveniles (French, 2021). Age-resistance to parasitic infections has been documented in other avian species (Blakeney & Dimmick, 1971; Nowicki et al., 1995) and in kiwi suffering from coccidial infections (Morgan et al., 2014). While the samples in this study were mostly from younger kiwi, other investigations (French, 2021) into this disease indicate similar findings.

Although most cases in young birds did not have very high EPG counts (median of 6 EPG) it is worth noting the presence of extreme outliers. A kiwi that excretes a high number of infective eggs into an environment shared with ther kiwi increases the chances of infection transmission compared to a kiwiwith a low EPG burden. The creche rearing system currently employed for raising kiwi in captive institutions often houses more than one kiwi in a given enclosure. Most kiwi in such locations are reared with the intention of release into the wild after attaining suitable weight (Germano et al., 2018) meaning that they are

typically young. This is reflected in the samples obtained from this study which are biased towards kiwi less than six months in age. Such juvenile birds have naïve immune systems. The presence of a sick bird with a high EPG count in a creche that houses other kiwi of similar immunological naivety could result in quick transmission of the disease. Further, as these creches are reused every season, a healthy flock of kiwi housed in a pen witha high EPG of soil substrate from previous years could all contract infection.

It is, however, interesting to note that all three of the extreme cases encountered in this study did not result in host mortality while some kiwi with much lower EPG than these have succumbed (pers obs). Whether morbidity is not a direct predictor of mortality or if parasite load in the faeces does not correlate to nematode burden in the ventriculus, cannot be accurately determined from these few cases. What can be said is that regardless of the quantitative burden of these nematode eggs, their mere presence should be considered potentially clinically significant and treated with caution. Similarly, cases identified as positive from this study were treated at their locations withanthelminthics.

The influence of sample location on disease prevalence could not be established. Had one submission location suffered from a higher disease prevalence that others, it would have implied that the environment at that particular location was uniquely suited to the parasite's lifecycle and transmission. While younger birds are more immunologically susceptible to infection than mature adults, the juvenile

birds examined in this study had all been born and raised in captive institutions. In other words, their environment had remained relatively unchanged throughout their lives thus far. As *Cyrnea* sp. and other spirurids require an intermediate host to complete their lifecycle,(Christie et al., 2011; Mejia-Fava et al., 2013; Niemuth et al., 2013) the ability of this parasite to cause patent infections in these captive birds indicates that the intermediate host required is present in the same environment as the hosts. This means that an unchanged environment would allow for the continued propagation of the parasite lifecycle and consequently, infection in kiwi. As positive samples were identified from several submission locations, the conditions required for disease transmission are present in several locations across New Zealand. Further, as the intermediate host required is unknown, (French, 2021; Zhang et al., 2004) its exact habitat (soil, air, water, etc.) is also unknown. This means that the key environmental feature required for the intermediate host (and by extension, the parasite) to successfully thrive cannot be identified at this time. This makes environmental control of the disease problematic. Further studies on the environmental transmission of the parasite are needed in order to examine the effect of location on the disease.

4.7. Limitations

This study was limited in its sampling and experimentation. While a large number of samples across many species and locations were included in

this study, a subset of these had to be excluded from analysis due to insufficient information regarding the host species, age or submission location.Further, despite nine submission locations participating in this study, the sampling was highly skewed with the bulk of the samples originating from the first three locations (A-C) and the other locations contributing too few samples to accurately analyse. The same can be said in terms of host species as very few samples of rowi and great spotted kiwi were obtained while no samplesfrom little spotted or Haast tokoeka kiwi were available for analysis. A moreholistic study would have been possible had these deficiencies been avoided.The method of testing used in this study was a faecal flotation in 80%zinc sulphate solution. While this is no longer considered the recommended test for quantification of VNK, it was the best test available at the time. In this light, future prevalence studies could opt for a centrifugal sedimentation in 33% zinc sulphate solution for their experimental methodology.

It is also worth noting that spirurid nematodes similar in morphology to *Cyrnea* sp. have been identified from the proventriculus and small intestines of kiwi from post-mortem investigations. The exact species of these worms have not been identified yet. However, the eggs resemble those of *Cyrnea* sp. in coprological investigations (French, 2021). This means that coprological diagnosis cannot accurately determine if the nematodes are present in the ventriculus or other locations in the GIT. However, these infections involving spirurids from the proventriculus and small intestine of kiwi were present at far lower

prevalence based on the prospective post-mortem study and no cases of clinical significance have yet been reported. This means that identification of a spirurid egg in the faeces of kiwi is most likely the result of VNK. Further, regardless of the origin of the nematode from the GIT, the clinical signs would be similar while treatment and control measures would remain the same. This distinction is important from a scientific perspective but it does not have much practical significance.

4.8. Conclusions

Much work remains to be done regarding the parasitic diseases of kiwi and their prevalence. This is especially true for nematodiases. To date there have been two studies regarding the helminthic diseases of kiwi. One study was retrospective and the other prospective, but both utilized post-mortem sampling for their experimentation. This is the first ante-mortem study on the prevalence of VNK that utilizes faecal sampling and coprological examination.Four-hundred and fifty samples were collected from captive kiwi housing facilities over the course of eight months. These samples were obtained from167 individual kiwi of four different subspecies across a wide age-range from nine different submission locations. The samples were analysed using faecal flotation in 80% zinc sulphate solution. Those samples that had at least one EPG were considered positive for infection. While this test is not 100% sensitive, it was the most reliable of those available at the time. The data revealed that the prevalence of VNK in captive kiwi is 16.77% (95% CI)

basedon faecal examinations. The study also demonstrated significant effects of host age and species on disease prevalence. Kiwi aged 4 months or youngerand those of the NIB species are the most likely to harbour infection. No significant relationship between submission locations and disease prevalence could be established. The study also revealed that while most infections are likely to produce egg counts of less than 60 EPG, (as determined by flotation in 80% zinc sulphate solution) cases which have extremely high parasite loadsin the faeces are also encountered. Regardless of the quantitative parasite load, it is recommended that all cases must be treated with caution and that clinicians and those associated with the captive kiwi rearing program should be on the lookout for potential infection in their younger birds.

4.9. References

Blakeney, W. C., & Dimmick, R. W. (1971). Gizzard and intestinal helminths of bobwhite quail in Tennessee. *The Journal of Wildlife Management, 35*(3), 4.

Christie, J., Schwan, E. V., Bodenstein, L. L., Sommerville, J. E. M., & van der Merwe, L. L. (2011). The sensitivity of direct faecal examination, direct faecal flotation, modified centrifugal faecal flotation and centrifugal sedimentation/flotation in the diagnosis of canine spirocercosis. *Joiurnal of the South African Veterinary Association, 82*, 6.

Doneley, B. (2006). Management of captive ratities. In G. Harrison & T. Lightfoot (Eds.), *Clinical Avian Medicine* (Vol. 2, pp. 957-989). Spix Publishing.

French, A. (2021). *A prospective survey of gastrointestinal nematodes in kiwi (Apteryx spp.)* [Thesis chapter]. Massey University.

French, A. F., Castillo-Alcala, F., Gedye, K. R., Knox, M. A., Roe, W. D., & Gartrell, B. D. (2020, Dec). Ventral dermatitis in rowi (Apteryx rowi) caused by cutaneous capillariasis. *Int J Parasitol Parasites Wildl, 13*, 160-170. https://doi.org/10.1016/j.ijppaw.2020.10.003

French, A. F., Castillo-Alcala, F., Gedye, K. R., Roe, W. D., & Gartrell, B. D. (2020, Apr). Nematode larva migrans caused by Toxocara cati in the North Island brown kiwi (Apteryx mantelli). *Int J Parasitol Parasites Wildl, 11*, 221-228. https://doi.org/10.1016/j.ijppaw.2020.02.011

Mejia-Fava, j., Divers, S. J., Jimenez, D. A., Ambrose, D. L., Rech, R., Gottdenker, N. L., & Mayer, J. (2013). Diagnosis and treatment of proventricular nematodiases in an umbrella cockatoo. *Journal of American Veterinary Medical Association, 242*, 5.

Morgan, K. J., Alley, M. R., Pomroy, W. E., Castro, I., & Howe, L. (2012, Oct). Enteric coccidiosis in the brown kiwi (Apteryx mantelli). *Parasitol Res, 111*(4), 1689-1699. https://doi.org/10.1007/s00436-012-3008-5

Morgan, K. J., Castro, I., Lopez-Villalobos, N., Pomroy, W. E., Alley, M. R., Gartrell, B. D., Hunter, S., & Howe, L. (2014, Nov). Prevalence of and risk factors for coccidiosis in kiwi between 1977 and 2011. *N Z Vet J, 62*(6), 315-320. https://doi.org/10.1080/00480169.2014.932657

Niemuth, J. N., Allgood, J. V., Flowers, J. R., De Voe, R. S., & Troan, B. V. (2013). Ventricular Habronemiasis in Aviary Passerines. *Case Reports in Veterinary Medicine, 2013*, 1-6. https://doi.org/10.1155/2013/719465

Nowicki, A., Roby, D. D., & Woolf, A. (1995). Gizzard nematodes of Canada geese wintering in southern Illinois. *Journal of wildlife disease, 31*(3), 7.

Taylor, H. S., Morgan, K. J., Pomroy, W. E., & McInnes, K. (2019, Mar). Apparent lack of efficacy of toltrazuril against Eimeria species affecting brown kiwi (Apteryx mantelli) at a captive rearing facility. *N Z Vet J, 67*(2), 101-104. https://doi.org/10.1080/00480169.2018.1541425

van Zyl, N. (2014). *Nematodiasis and larval migrans in kiwi (Apteryx spp.) : a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Zoology at Massey University, Palmerston North, New Zealand* [Masters, Massey University]. http://hdl.handle.net/10179/6322

Zhang, L., Brooks, D. R., & Causey, D. (2004). Procyrnea Chabaud 1958 (Nematoda: habrnonemtoidea: habronematidae) in birds form the area de conservacion guanacaste, Costa Rica, including desciptions of 3 new species. *Journal of Parasitology, 90*, 10.

5 General Discussion

Ventricular nematodiasis in kiwi (VNK) has been identified as a potential, previously unrecognised cause of morbidity in kiwi in recent years (French, 2021). The causative agent is believed to be a spirurid nematode of the genus *Cyrnea* sp. (French, 2021). Chapter 2 of this thesis examines four such cases, two of which resulted in mortality of the kiwi. The clinical signs of the disease were identified as melaena/haematochezia, weight loss and inappetence or anorexia. These are non-specific signs associated with several parasitic and non-parasitic diseases of the GIT. As such, they alone cannot be used to make an accurate diagnosis. This has been historically achieved through gastroscopic evaluation of the ventriculus and necropsy examination (French, 2021). While both these methods provide an accurate diagnosis, post-mortem examination cannot be applied to clinical cases. Performing gastroscopy on young kiwi may not be possible depending on the size of bird and the scope available. At Wildbase hospital, gastroscopy is limited to kiwi weighing 800g or more (Jolly, personal communication, 2020) as the scope available (7.9 mm diameter flexible scope) cannot be passed to the level of the ventriculus in smaller birds. An ante-mortem test that could be applied to all kiwi for accurate diagnosis of ventricular nematodiasis was lacking. This is especially concerning given that chapter 2 of this thesis establishes ventricular nematodiasis as a cause of morbidity and mortality in kiwi.

Parasitic diseases of the gastrointestinal tract in human and

veterinary medicine are routinely diagnosed using coprological examination (Thienpont et al., 1986). In kiwi, a 33% centrifugal flotation is used for routine diagnosis ofcoccidiosis and helminth infections such as *Heterakis* sp. (Morgan, 2008, 2013). However, coprological testing has failed to detect the parasite responsible for VNK for several years (French, 2021). Different methods of coproscopy and several solutions were tested to determine an ante-mortem faecal test capable of diagnosing VNK. Opportunistic sampling of kiwi faeces for nine months across several different captive institutions were utilised in this prospective study. Faecal flotations in 60%, 68% and 80% zinc sulphate solutions and centrifugal sedimentation in 33% zinc sulphate solution were found to detect the eggs of *Cyrnea* sp. Of these tests, 60% and 68% flotation had poor detection rates compared to the other methods. Faecal flotation in 80% zinc sulphate was found to be the most sensitive method of the tests employed to detect VNK. However, sedimentation in 33% zinc sulphate was found to provide a higher egg per gram (EPG) count per sample. The latter test can also be used in conjunction with 33% flotation. This allows a single sample to be tested for coccidiosis, VNK and other helminth infections. This reduces the time and effort spent on sample preparation and the volume of sample required per test and is therefore more practical for the laboratory. In addition, laboratory personnel would not need any additional instruction or training. It is also cheaper than testing in 80% zinc sulphate solution. For these advantages, the recommended test method of routine screening for VNK

in laboratories may be centrifugal sedimentation in 33% zinc sulphate solution. However, owing to the higher sensitivity of flotation in 80% zinc sulphate, this test can be considered for cases suspected of VNK or for further study of the disease.

The prevalence of VNK in kiwi in captive housing facilities was found to be 16.77% (95% CI) using 80% faecal flotation. This was determined through a prospective study using opportunistic faecal sampling over a period of eight months using kiwi from locations across the country. This study also revealed that host age and species had significant effects on the disease prevalence. In this study VNK was found most commonly in young North Island brown (NIB) kiwi aged between 3-4 months and in the Eastern (EB) genotype. A significant relationship between VNK and captive facility location could not be established, however the parasite was found in most of the sampling locations. In other words, VNK is present throughout New Zealand.

Although nematodes of the genus *Cyrnea* have been found in the small intestine and proventriculus of kiwi (French, 2021), as mentioned in chapter 4 of this thesis, their incidence was found to be considerably lower than spirurids infestations of the kiwi ventriculus. Further, it is worth noting that all identified cases of small intestinal and proventricular spirurid infestations were seen in association with ventricular spirurid infestations by *Cyrnea* sp in much higher numbers (French, 2021). Therefore, spirurids identified by coprological investigations of kiwi faeces are likely to have originated either solely or conjointly from the

ventriculus. In other words, despite the identification of spirurid nematodes from the small intestine and proventriculus their association with VNK and the lack of clinical disease associated with extra-ventricular nematodiases justify the use of this term for clarity, at least in the clinical setting.

For kiwi conservation management, the Operation Nest Egg (ONE) project rears kiwi chicks from hatch until they attain a stoat-resistant weight of about one kilogram (Germano et al., 2018). The captive management programme for kiwi houses mostly the EB genotype of the NIB kiwi (Barlow, 2018). This means that majority of the kiwi in captive housing locations are young NIB, i.e., the most likely to suffer from VNK. This information, coupled with the prevalence data obtained in this thesis warrant that those involved in the captive kiwi breeding and housing programmes must consider and manage for VNK. This extends to not only the conservation organisations involved, but also local wildlife hospitals and diagnostic laboratories.

Perhaps the most important aspect of monitoring for VNK is to utilise routine coprological testing of susceptible kiwi and ensure that diagnostic laboratories use a method of examination that will detect the relatively heavy ova of these parasites. The logistical requirements for this would be few and the protocol for sampling and laboratory examination has been established through this study. It is not unreasonable to assume that these measures would be easy to follow regularly as they could easily be added to the ongoing surveillance for

kiwi coccidiosis (Morgan, 2013; Morgan et al., 2012).

Another control measure that would potentially reduce VNK is to ensure that substrate is regularly turned-over or replaced in creche rearing sites and communal outdoor pens. This technique is also recommended for the prevention of coccidiosis but is only variably applied by rearing facilities due to cost and logistical difficulty. Coccidiosis is thought to occur in higher prevalence in captive locations compared to free living/wild kiwi populations due to the intensive stocking of young, immunologically naïve kiwi that share a common environment. As the parasite is excreted in the faeces, the soil in such locations may also contain a high parasite burden which can increase the likelihood of transmission (Morgan, 2013; Morgan et al., 2012; Morgan et al., 2014). These conditions that favour the spread of coccidiosis may also favour the spread of VNK. Rotation of the kiwi into clean houses as well as spelling and turnover of housing substrate may help impede the parasite lifecycle and transmission. However, these would require considerable increases in the space available for kiwi rearing projects.

An effective control measure against VNK would be to interfere with the parasite lifecycle by targeting the intermediate host. However, this would require more detailed information regarding the lifecycle of the parasite than is currently available, including the identification of possible intermediate hosts. *Cyrnea apterycis* was identified from kiwi several decades ago, (Harris, 1975) but there has since been the suggestion that several different *Cyrnea* species may parasitise kiwi

(French 2021). Recent studies concur that the nematodes commonly found in the ventriculus of kiwi are morphologically consistent with members of the *Cyrnea* genus as previously described, (French, 2021) but an exact identification has not been reached. Preliminary molecular evaluation was limited by a lack of comparative sequence material available in genomic databases (French, 2021). Further study of these nematodes would therefore benefit from both expert taxonomic examination and more extensive genetic studies. Most of the knowledge of this parasite comes from studies of related genera like *Procyrnea* and the only information regarding its lifecycle comes from extrapolating knowledge of similar parasites' lifecycles (Zhang et al., 2004; Zhang et al., 2011). The proper identification of the causative agent of VNK and the details of its lifecycle are important gaps in our understanding of this disease and how to control it.

The diagnostic tests developed during this research project are more accurate and sensitive compared to previously available tests. But the test which is most sensitive, is not accurate and the test most accurate, is not as sensitive. A gold standard test that can detect the true parasite load per antemortem sample may not be possible given the inherent insensitivity of coprological examination (Christie et al., 2011). However, a test that combines or exceeds the sensitivity of faecal flotation in 80% zinc sulphate with the accuracy of sedimentation in 33% zinc sulphate would be a step in this direction. Such a test would not force prioritising sensitivity over accuracy or vice versa. Of the currently

available methods, the most practical screening test is proposed to be centrifugal sedimentation in 33% zinc sulphate solution. Further refinement of the currently available tests or the discovery of a new test altogether is still a promising area for future research to explore.

Another area of possible future work would be investigation of the disease patterns. While the prevalence of VNK was determined in captive kiwi faecal samples using centrifugal flotation in 80% zinc sulphate solution, this study was opportunistic in its sampling and may not represent the true prevalence. The samples obtained were also largely from juvenile NIB kiwi less than six months in age. This is because captive housing facilities largely focus on this age group and species. The prevalence in other age ranges and species from captive institutions were examined in a limited capacity in this thesis but further sampling from such individuals could alter the current prevalence estimates. Further, the prevalence of the disease in wild populations is still unknown. This study could not establish a relationship between disease occurrence and sampling location. As discussed in chapter 4, such a relationship could have implications on the transmission and lifecycle of the parasite. Future studies could choose to focus on this aspect of the host-parasite-environment dynamic.

In conclusion, this study has provided strong evidence that VNK is a common and important disease of captive kiwi and has helped to refine the diagnostic tests available to detect the presence of the parasite in live kiwi. The captive management of kiwi for conservation

has likely resulted in the multiplication of this parasite and magnification of its pathogenic effects, but further study will be needed to confirm this in relation to wild kiwi populations.

5.1. References

Christie, J., Schwan, E. V., Bodenstein, L. L., Sommerville, J. E. M., & van der Merwe, L. L. (2011). The sensitivity of direct faecal examination, direct faecal flotation, modified centrifugal faecal flotation and centrifugal sedimentation/flotation in the diagnosis of canine spirocercosis. *Joiurnal of the South African Veterinary Association, 82*, 6.

French, A. (2021). *A prospective survey of gastrointestinal nematodes in kiwi (Apteryx spp.)* [Thesis chapter]. Massey University.

Harris. (1975). Two New Nematodes Parasitic in the Kiwi in New Zealand. *Bulletin of the British Museum*, 7.

Morgan, K. J. (2008). *Kiwi first aid and veterinary care*. Department of Conservation.

Morgan, K. J. (2013). *Coccidiosis in the kiwi (Apteryx spp.) : aspects of the pathology, epidemiology and parasite biology : a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Veterinary Science at Massey University, Palmerston North, New Zealand* [Doctoral, Massey University]. http://hdl.handle.net/10179/6340

Morgan, K. J., Alley, M. R., Pomroy, W. E., Castro, I., & Howe, L. (2012, Oct). Enteric coccidiosis in the brown kiwi (Apteryx mantelli). *Parasitol Res, 111*(4), 1689-1699. https://doi.org/10.1007/s00436-012-3008-5

Morgan, K. J., Castro, I., Lopez-Villalobos, N., Pomroy, W. E., Alley, M. R., Gartrell, B. D., Hunter, S., & Howe, L. (2014, Nov). Prevalence of and risk factors for coccidiosis in kiwi between 1977 and 2011. *N Z Vet J, 62*(6), 315-320. https://doi.org/10.1080/00480169.2014.932657

Thienpont, D., Rochette, F., & Vanparijs, O. F. J. (1986). *Diagnosing helminthiasis by coprological examination* (Vol. 1986). Janssen Research Foundation Beerse, Belgium.

Zhang, L., Brooks, D. R., & Causey, D. (2004). Procyrnea Chabaud 1958 (Nematoda: habrnonemtoidea: habronematidae) in birds form the area de conservacion guanacaste, Costa Rica, including desciptions of 3 new species. *Journal of Parasitology, 90*, 10.

Zhang, S., Song, J., & Zhang, L. (2011). Three species ofProcyrneaChabaud, 1958 (Nematoda: Habronematoidea: Habronematidae) from raptors in Beijing, China, with descriptions of two new species. *Journal of Natural History, 45*(47-48), 2915-2928. https://doi.org/10.1080/00222933.2011.622451

Appendix 1 Results of coprological examination of kiwi faecal samples in 80% zinc sulphate flotation

Sample number	Kiwi number	Species	Submitter	Age range	Result	EPG	Sample number	Kiwi number	Species	Submitter	Age range	Result	EPG
1.	1	NIB	A	3-4 months	Neg	0	231.	80	EB	C	3-4 months	Neg	0
2.	1	NIB	A	3-4 months	Neg	0	232.	80	EB	C	3-4 months	Neg	0
3.	1	NIB	A	3-4 months	Neg	0	233.	80	EB	C	3-4 months	Neg	0
4.	1	NIB	A	3-4 months	Neg	0	234.	80	EB	C	3-4 months	Neg	0
5.	2	NIB	A	3-4 months	Neg	0	235.	80	EB	C	3-4 months	Neg	0
6.	3		B	< 3 months	Neg	0	236.	80	EB	C	3-4 months	Neg	0
7.	4	EB	C	1-5 years	Neg	0	237.	80	EB	C	3-4 months	Neg	0
8.	4	EB	C	1-5 years	Neg	0	238.	80	EB	C	3-4 months	Neg	0
9.	4	EB	C	1-5 years	Neg	0	239.	81	NIB	A	3-4 months	Neg	0
10.	5	EB	B	< 3 months	Neg	0	240.	81	NIB	A	3-4 months	Neg	0
11.	5	EB	B	< 3 months	Neg	0	241.	81	NIB	A	3-4 months	Neg	0
12.	5	EB	B	< 3 months	Neg	0	242.	82	NIB	A	3-4 months	Neg	0
13.	5	EB	B	< 3 months	Neg	0	243.	82	NIB	A	3-4 months	Neg	0
14.	5	EB	B	< 3 months	Neg	0	244.	83	NIB	D	+ 5 years	Neg	0
15.	6	Rowi	D	3-4 months	Neg	0	245.	83	NIB	D	+ 5 years	Neg	0
16.	6	Rowi	D	3-4 months	Neg	0	246.	84	EB	B	< 3 months	Neg	0
17.	6	Rowi	D	3-4 months	Neg	0	247.	84	EB	B	< 3 months	Neg	0
18.	6	Rowi	D	3-4 months	Neg	0	248.	85	NIB	A	3-4 months	Neg	0
19.	7	Rowi	D	3-4 months	Neg	0	249.	86		B	< 3 months	Neg	0
20.	8	NIB	A	3-4 months	Neg	0	250.	86		B	< 3 months	Neg	0
21.	8	NIB	A	3-4 months	Neg	0	251.	87	EB	B	< 3 months	Neg	0
22.	8	NIB	A	3-4 months	Neg	0	252.	87	EB	B	< 3 months	Neg	0
23.	8	NIB	A	3-4 months	Neg	0	253.	87	EB	B	< 3 months	Neg	0

24.	8	NIB	A	3-4 months	Neg	0		254.	87	EB	B	< 3 months	Neg	0
25.	9	NIB	A	3-4 months	Neg	0		255.	88	NIB	E	< 3 months	Neg	0
26.	9	NIB	A	3-4 months	Neg	0		**256.**	**89**	**NIB**	**I**	**3-4 months**	**Pos**	**4**
27.	**9**	**NIB**	**A**	**3-4 months**	**Pos**	**50**		257.	90	NIB	I	3-4 months	Neg	0
28.	9	NIB	A	3-4 months	Neg	0		258.	91	NIB	A	3-4 months	Neg	0
29.	**9**	**NIB**	**A**	**3-4 months**	**Pos**	**12**		259.	92	NIB	A	3-4 months	Neg	0
30.	10	NIB	A	3-4 months	Neg	0		260.	92	NIB	A	3-4 months	Neg	0
31.	11	NIB	D	1-5 years	Neg	0		261.	92	NIB	A	3-4 months	Neg	0
32.	11	NIB	D	1-5 years	Neg	0		262.	92	NIB	A	3-4 months	Neg	0
33.	12	NIB	A	3-4 months	Neg	0		263.	93	NIB		< 3 months	Neg	0
34.	**12**	**NIB**	**A**	**3-4 months**	**Pos**	**12**		**264.**	**93**	**NIB**		**< 3 months**	**Pos**	**8**
35.	12	NIB	A	3-4 months	Neg	0		265.	94				Neg	0
36.	12	NIB	A	3-4 months	Neg	0		266.	95	NIB	F		Neg	0
37.	13	NIB	E	< 3 months	Neg	0		267.	96	NIB	A	3-4 months	Neg	0
38.	14	NIB	D	3-4 months	Neg	0		268.	97	NIB	E	< 3 months	Neg	0
39.	15	EB	C	+ 5 years	Neg	0		269.	97	NIB	E	< 3 months	Neg	0
40.	16	NIB	A	3-4 months	Neg	0		270.	98	NIB	A	3-4 months	Neg	0
41.	16	NIB	A	3-4 months	Neg	0		271.	98	NIB	A	3-4 months	Neg	0
42.	16	NIB	A	3-4 months	Neg	0		**272.**	**98**	**NIB**	**A**	**3-4 months**	**Pos**	**60**
43.	17	NIB	A	3-4 months	Neg	0		273.	99	NIB	D	+ 5 years	Neg	0
44.	17	NIB	A	3-4 months	Neg	0		274.	99	NIB	D	+ 5 years	Neg	0
45.	17	NIB	A	3-4 months	Neg	0		**275.**	**100**	**NIB**	**A**	**3-4 months**	**Pos**	**12**
46.	17	NIB	A	3-4 months	Neg	0		**276.**	**100**	**NIB**	**A**	**3-4 months**	**Pos**	**4**
47.	17	NIB	A	3-4 months	Neg	0		277.	100	NIB	A	3-4 months	Neg	0

48.	17	NIB	A	3-4 months	Neg	0	278.	100	NIB	A	3-4 months	Neg	0
49.	17	NIB	A	3-4 months	Neg	0	279.	100	NIB	A	3-4 months	Neg	0
50.	17	NIB	A	3-4 months	Neg	0	280.	100	NIB	A	3-4 months	Neg	0
51.	17	NIB	A	3-4 months	Neg	0	281.	101	NIB	H	6-12 months	Neg	0
52.	17	NIB	A	3-4 months	Neg	0	282.	102	NIB	A	3-4 months	Neg	0
53.	17	NIB	A	3-4 months	Neg	0	283.	103	NIB	A	3-4 months	Neg	0
54.	18	NIB	A	3-4 months	Neg	0	**284.**	**103**	**NIB**	**A**	**3-4 months**	**Pos**	**36**
55.	18	NIB	A	3-4 months	Neg	0	285.	104	NIB	A	3-4 months	Neg	0
56.	18	NIB	A	3-4 months	Neg	0	286.	104	NIB	A	3-4 months	Neg	0
57.	**18**	**NIB**	**A**	**3-4 months**	**Pos**	**68**	287.	104	NIB	A	3-4 months	Neg	0
58.	**18**	**NIB**	**A**	**3-4 months**	**Pos**	**4**	288.	104	NIB	A	3-4 months	Neg	0
59.	19	EB	B	< 3 months	Neg	0	289.	104	NIB	A	3-4 months	Neg	0
60.	19	EB	B	< 3 months	Neg	0	290.	104	NIB	A	3-4 months	Neg	0
61.	20	NIB	A	3-4 months	Neg	0	291.	104	NIB	A	3-4 months	Neg	0
62.	**20**	**NIB**	**A**	**3-4 months**	**Pos**	**2**	292.	105	NIB	A	3-4 months	Neg	0
63.	20	NIB	A	3-4 months	Neg	0	293.	106	NIB	A	3-4 months	Neg	0
64.	21	Rowi	D	3-4 months	Neg	0	**294.**	**107**	**NIB**	**A**	**3-4 months**	**Pos**	**2**
65.	22	NIB	A	3-4 months	Neg	0	**295.**	**107**	**NIB**	**A**	**3-4 months**	**Pos**	**24**
66.	22	NIB	A	3-4 months	Neg	0	**296.**	**107**	**NIB**	**A**	**3-4 months**	**Pos**	**20**
67.	**22**	**NIB**	**A**	**3-4 months**	**Pos**	**22**	297.	108	Greater spotted	D	+ 5 years	Neg	0
68.	23	Rowi	D	3-4 months	Neg	0	298.	108	Greater spotted	D	+ 5 years	Neg	0
69.	**24**	**NIB**	**A**	**3-4 months**	**Pos**	**6**	299.	109	EB	B	< 3 months	Neg	0
70.	**24**	**NIB**	**A**	**3-4 months**	**Pos**	**128**	300.	109	EB	B	< 3 months	Neg	0

71.	**24**	**NIB**	**A**	**3-4 months**	**Pos**	**10**		301.	109	EB	B	< 3 months	Neg	0
72.	**25**	**EB**	**B**	**< 3 months**	**Pos**	**4**		302.	110	NIB	A	3-4 months	Neg	0
73.	25	EB	B	< 3 months	Neg	0		303.	110	NIB	A	3-4 months	Neg	0
74.	26			?	Neg	0		304.	110	NIB	A	3-4 months	Neg	0
75.	27			?	Neg	0		305.	111	NIB	A	3-4 months	Neg	0
76.	28	NIB	A	3-4 months	Neg	0		306.	111	NIB	A	3-4 months	Neg	0
77.	28	NIB	A	3-4 months	Neg	0		307.	111	NIB	A	3-4 months	Neg	0
78.	28	NIB	A	3-4 months	Neg	0		308.	112	NIB	A	3-4 months	Neg	0
79.	29	NIB	A	3-4 months	Neg	0		**309.**	**113**	**NIB**	**A**	**3-4 months**	**Pos**	**202**
80.	30	NIB	A	3-4 months	Neg	0		310.	113	NIB	A	3-4 months	Neg	0
81.	30	NIB	A	3-4 months	Neg	0		311.	113	NIB	A	3-4 months	Neg	0
82.	31	NIB	A	3-4 months	Neg	0		312.	114	EB	C	3-4 months	Neg	0
83.	31	NIB	A	3-4 months	Neg	0		313.	115		E	< 3 months	Neg	0
84.	31	NIB	A	3-4 months	Neg	0		314.	116	EB	B	< 3 months	Neg	0
85.	31	NIB	A	3-4 months	Neg	0		315.	117	EB	C	1-5 years	Neg	0
86.	**31**	**NIB**	**A**	**3-4 months**	**Pos**	**6**		316.	117	EB	C	1-5 years	Neg	0
87.	31	NIB	A	3-4 months	Neg	0		317.	118	NIB	D	1-5 years	Neg	0
88.	31	NIB	A	3-4 months	Neg	0		318.	118	NIB	D	1-5 years	Neg	0
89.	31	NIB	A	3-4 months	Neg	0		319.	119	EB	B	< 3 months	Neg	0
90.	32	NIB	E	< 3 months	Neg	0		320.	119	EB	B	< 3 months	Neg	0
91.	33	NIB	D	?	Neg	0		321.	119	EB	B	< 3 months	Neg	0
92.	33	NIB	D	?	Neg	0		322.	119	EB	B	< 3 months	Neg	0
93.	34	NIB	A	3-4 months	Neg	0		323.	119	EB	B	< 3 months	Neg	0
94.	34	NIB	A	3-4 months	Neg	0		324.	119	EB	B	< 3 months	Neg	0

95.	34	NIB	A	3-4 months	Neg	0		325.	120	NIB	A	3-4 months	Neg	0
96.	34	NIB	A	3-4 months	Neg	0		326.	120	NIB	A	3-4 months	Neg	0
97.	34	NIB	A	3-4 months	Neg	0		327.	120	NIB	A	3-4 months	Neg	0
98.	34	NIB	A	3-4 months	Neg	0		328.	120	NIB	A	3-4 months	Neg	0
99.	35	Rowi	D	< 3 months	Neg	0		329.	120	NIB	A	3-4 months	Neg	0
100.	36	Rowi	D	< 3 months	Neg	0		330.	120	NIB	A	3-4 months	Neg	0
101.	36	Rowi	D	< 3 months	Neg	0		331.	120	NIB	A	3-4 months	Neg	0
102.	37		B	< 3 months	Neg	0		332.	121	NIB	A	3-4 months	Neg	0
103.	37		B	< 3 months	Neg	0		333.	121	NIB	A	3-4 months	Neg	0
104.	38	EB	B	< 3 months	Neg	0		334.	121	NIB	A	3-4 months	Neg	0
105.	38	EB	B	< 3 months	Neg	0		335.	121	NIB	A	3-4 months	Neg	0
106.	38	EB	B	< 3 months	Neg	0		336.	121	NIB	A	3-4 months	Neg	0
107.	38	EB	B	< 3 months	Neg	0		337.	122	NIB	D	1-5 years	Neg	0
108.	38	EB	B	< 3 months	Neg	0		338.	123	NIB		< 3 months	Neg	0
109.	38	EB	B	< 3 months	Neg	0		339.	124	NIB	D	6-12 months	Neg	0
110.	38	EB	B	< 3 months	Neg	0		340.	125	NIB	D	6-12 months	Neg	0
111.	39			< 3 months	Neg	0		341.	125	NIB	A	3-4 months	Neg	0
112.	40	NIB	A	3-4 months	Neg	0		342.	126	NIB	A	3-4 months	Neg	0
113.	41	NIB	E	< 3 months	Neg	0		343.	126	NIB	A	3-4 months	Neg	0
114.	42	EB	B	< 3 months	Neg	0		344.	126	NIB	A	3-4 months	Neg	0
115.	42	EB	B	< 3 months	Neg	0		345.	126	NIB	A	3-4 months	Neg	0
116.	42	EB	B	< 3 months	Neg	0		346.	126	NIB	A	3-4 months	Neg	0
117.	42	EB	B	< 3 months	Neg	0		347.	127	NIB	A	3-4 months	Neg	0
118.	43	NIB	A	3-4 months	Neg	0		348.	127	NIB	A	3-4 months	Neg	0

119.	43	NIB	A	3-4 months	Neg	0		349.	127	NIB	A	3-4 months	Neg	0
120.	43	NIB	A	3-4 months	Neg	0		350.	128	NIB	A	3-4 months	Neg	0
121.	43	NIB	A	3-4 months	Neg	0		351.	128	NIB	A	3-4 months	Neg	0
122.	43	NIB	A	3-4 months	Neg	0		352.	128	NIB	A	3-4 months	Neg	0
123.	**43**	**NIB**	**A**	**3-4 months**	**Pos**	**44**		353.	128	NIB	A	3-4 months	Neg	0
124.	43	NIB	A	3-4 months	Neg	0		354.	129	NIB	E	< 3 months	Neg	0
125.	43	NIB	A	3-4 months	Neg	0		355.	130	EB	B	< 3 months	Neg	0
126.	**43**	**NIB**	**A**	**3-4 months**	**Pos**	**2**		356.	130	EB	B	< 3 months	Neg	0
127.	43	NIB	A	3-4 months	Neg	0		357.	130	EB	B	< 3 months	Neg	0
128.	43	NIB	A	3-4 months	Neg	0		358.	130	EB	B	< 3 months	Neg	0
129.	43	NIB	A	3-4 months	Neg	0		359.	131	EB	B	< 3 months	Neg	0
130.	43	NIB	A	3-4 months	Neg	0		360.	131	EB	B	< 3 months	Neg	0
131.	43	NIB	A	3-4 months	Neg	0		361.	131	EB	B	< 3 months	Neg	0
132.	43	NIB	A	3-4 months	Neg	0		362.	131	EB	B	< 3 months	Neg	0
133.	43	NIB	A	3-4 months	Neg	0		363.	131	EB	B	< 3 months	Neg	0
134.	43	NIB	A	3-4 months	Neg	0		364.	132	NIB	D	1-5 years	Neg	0
135.	43	NIB	A	3-4 months	Neg	0		365.	132	NIB	D	1-5 years	Neg	0
136.	44	EB	C	+ 5 years	Neg	0		366.	133	NIB	B	< 3 months	Neg	0
137.	44	EB	C	+ 5 years	Neg	0		367.	133	NIB	B	< 3 months	Neg	0
138.	44	EB	C	+ 5 years	Neg	0		368.	134	EB	B	< 3 months	Neg	0
139.	44	EB	C	+ 5 years	Neg	0		369.	134	EB	B	< 3 months	Neg	0
140.	44	EB	C	+ 5 years	Neg	0		370.	134	EB	B	< 3 months	Neg	0
141.	**45**	**NIB**	**A**	**3-4 months**	**Pos**	**46**		371.	135	NIB	A	3-4 months	Neg	0
142.	45	NIB	A	3-4 months	Neg	0		372.	135	NIB	A	3-4 months	Neg	0

143.	45	NIB	A	3-4 months	Neg	0		373.	135	NIB	A	3-4 months	Neg	0
144.	46	EB	B	< 3 months	Neg	0		374.	136		E	< 3 months	Neg	0
145.	46	EB	B	< 3 months	Neg	0		375.	137	NIB	B	< 3 months	Neg	0
146.	46	EB	B	< 3 months	Neg	0		376.	138	NIB		< 3 months	Neg	0
147.	46	EB	B	< 3 months	Neg	0		377.	139	NIB	A	3-4 months	Neg	0
148.	47			?	Neg	0		378.	140	EB	C	+ 5 years	Neg	0
149.	48			?	Neg	0		379.	141	NIB			Neg	0
150.	49	EB	C	< 3 months	Neg	0		380.	142	NIB	A	3-4 months	Neg	0
151.	49	EB	C	< 3 months	Neg	0		381.	142	NIB	A	3-4 months	Neg	0
152.	49	EB	C	< 3 months	Neg	0		382.	142	EB	B	< 3 months	Neg	0
153.	49	EB	C	< 3 months	Neg	0		383.	142	EB	B	< 3 months	Neg	0
154.	49	EB	C	< 3 months	Neg	0		384.	143	NIB	A	3-4 months	Neg	0
155.	50	NIB	A	3-4 months	Neg	0		385.	143	NIB	A	3-4 months	Neg	0
156.	51	NIB	D	1-5 years	Neg	0		386.	143	NIB	A	3-4 months	Neg	0
157.	51	NIB	D	1-5 years	Neg	0		387.	143	NIB	A	3-4 months	Neg	0
158.	52	EB	B	3-4 months	Neg	0		388.	144	NIB	E	< 3 months	Neg	0
159.	52	EB	B	3-4 months	Neg	0		389.	145	NIB	E	< 3 months	Neg	0
160.	52	EB	B	3-4 months	Neg	0		390.	146	NIB	E	< 3 months	Neg	0
161.	53	EB	B	3-4 months	Neg	0		391.	147				Neg	0
162.	54	NIB	D	6-12 months	Neg	0		392.	147	NIB	A	3-4 months	Neg	0
163.	54	NIB	D	6-12 months	Neg	0		393.	147	NIB	A	3-4 months	Neg	0
164.	55	NIB	A	3-4 months	Neg	0		394.	147	NIB	A	3-4 months	Neg	0
165.	55	NIB	A	3-4 months	Neg	0		395.	147	NIB	A	3-4 months	Neg	0
166.	55	NIB	A	3-4 months	Neg	0		396.	148	EB	B	< 3 months	Neg	0

167.	55	NIB	A	3-4 months	Neg	0
168.	55	NIB	A	3-4 months	Neg	0
169.	56	NIB	F	?	Neg	0
170.	57	EB	C	< 3 months	Neg	0
171.	57	EB	C	< 3 months	Neg	0
172.	57	EB	C	< 3 months	Neg	0
173.	57	EB	C	< 3 months	Neg	0
174.	**58**	**NIB**	**A**	**3-4 months**	**Pos**	**8**
175.	58	NIB	A	3-4 months	Neg	0
176.	58	NIB	A	3-4 months	Neg	0
177.	58	NIB	A	3-4 months	Neg	0
178.	58	NIB	A	3-4 months	Neg	0
179.	58	NIB	A	3-4 months	Neg	0
180.	58	NIB	A	3-4 months	Neg	0
181.	58	NIB	A	3-4 months	Neg	0
182.	59	NIB	F	?	Neg	0
183.	60	NIB	F	?	Neg	0
184.	61		B	< 3 months	Neg	0
185.	62	EB	C	6-12 months	Neg	0
186.	62	EB	C	6-12 months	Neg	0
187.	62	EB	C	6-12 months	Neg	0
188.	62	EB	C	6-12 months	Neg	0
189.	63	Western Brown	B	< 3 months	Neg	0

397.	148	EB	B	< 3 months	Neg	0
398.	148	EB	B	< 3 months	Neg	0
399.	149	EB	B	< 3 months	Neg	0
400.	149	EB	B	< 3 months	Neg	0
401.	149	EB	B	< 3 months	Neg	0
402.	149	EB	B	< 3 months	Neg	0
403.	149	EB	B	< 3 months	Neg	0
404.	150	NIB	E	< 3 months	Neg	0
405.	150	NIB	E	< 3 months	Neg	0
406.	151				Neg	0
407.	152	EB	B	< 3 months	Neg	0
408.	152	EB	B	< 3 months	Neg	0
409.	152	EB	B	< 3 months	Neg	0
410.	152	EB	B	< 3 months	Neg	0
411.	152	EB	B	< 3 months	Neg	0
412.	153	NIB	E	< 3 months	Neg	0
413.	**153**	**NIB**	**E**	**< 3 months**	**Pos**	**8**
414.	154	EB	C	+ 5 years	Neg	0
415.	154	EB	C	+ 5 years	Neg	0
416.	154	EB	C	+ 5 years	Neg	0
417.	154	EB	C	+ 5 years	Neg	0
418.	155	EB	B	< 3 months	Neg	0
419.	155	EB	B	< 3 months	Neg	0

190.	63	Western Brown	B	< 3 months	Neg	0	420.	155	EB	B	< 3 months	Neg	0
191.	63	Western Brown	B	< 3 months	Neg	0	421.	156	NIB	A	3-4 months	Neg	0
192.	64	EB	B	< 3 months	Neg	0	422.	157	NIB	A	3-4 months	Neg	0
193.	64	EB	B	< 3 months	Neg	0	**423.**	**157**	**NIB**	**A**	**3-4 months**	**Pos**	**2**
194.	64	EB	B	< 3 months	Neg	0	424.	157	NIB	A	3-4 months	Neg	0
195.	64	EB	B	< 3 months	Neg	0	425.	157	NIB	A	3-4 months	Neg	0
196.	65	NIB		?	Neg	0	426.	157	NIB	A	3-4 months	Neg	0
197.	66	NIB		?	Neg	0	427.	157	NIB	A	3-4 months	Neg	0
198.	67	NIB	A	3-4 months	Neg	0	428.	157	NIB	A	3-4 months	Neg	0
199.	68	NIB	A	3-4 months	Neg	0	429.	158		E	< 3 months	Neg	0
200.	68	NIB	A	3-4 months	Neg	0	430.	159	EB	B	< 3 months	Neg	0
201.	69	EB	C	1-5 years	Neg	0	431.	159	EB	B	< 3 months	Neg	0
202.	70	NIB	D	+ 5 years	Neg	0	**432.**	**160**	**NIB**	**A**	**3-4 months**	**Pos**	**22**
203.	70	NIB	D	+ 5 years	Neg	0	433.	161	NIB	A	3-4 months	Neg	0
204.	**71**	**NIB**	**A**	**3-4 months**	**Pos**	**6**	**434.**	**161**	**NIB**	**A**	**3-4 months**	**Pos**	**100**
205.	72			?	Neg	0	435.	161	NIB	A	3-4 months	Neg	0
206.	**73**	**EB**	**B**	**< 3 months**	**Pos**	**2**	436.	161	NIB	A	3-4 months	Neg	0
207.	**73**	**EB**	**B**	**< 3 months**	**Pos**	**2**	437.	162	NIB	A	3-4 months	Neg	0
208.	73	EB	B	< 3 months	Neg	0	438.	163	NIB		< 3 months	Neg	0
209.	74	NIB	A	3-4 months	Neg	0	**439.**	**163**	**NIB**		**< 3 months**	**Pos**	**124**
210.	75	NIB	A	3-4 months	Neg	0	440.	164	EB	B	< 3 months	Neg	0
211.	75	NIB	A	3-4 months	Neg	0	441.	164	EB	B	< 3 months	Neg	0
212.	75	NIB	A	3-4 months	Neg	0	442.	165	NIB	A	3-4 months	Neg	0

213.	75	NIB	A	3-4 months	Neg	0			443.	165	NIB	A	3-4 months	Neg	0
214.	75	NIB	A	3-4 months	Neg	0			**444.**	**165**	**NIB**	**A**	**3-4 months**	**Pos**	**64**
215.	75	NIB	A	3-4 months	Neg	0			445.	166	NIB	A	3-4 months	Neg	0
216.	75	NIB	A	3-4 months	Neg	0			**446.**	**166**	**NIB**	**A**	**3-4 months**	**Pos**	**10**
217.	76	Rowi	D	< 3 months	Neg	0			447.	166	NIB	A	3-4 months	Neg	0
218.	76	Rowi	D	< 3 months	Neg	0			448.	167	Haast	G		Neg	0
219.	76	Rowi	D	< 3 months	Neg	0			449.	167	Haast	G		Neg	0
220.	77	EB	C	1-5 years	Neg	0			450.	167	Haast	G		Neg	0
221.	77	EB	C	1-5 years	Neg	0									
222.	77	EB	C	1-5 years	Neg	0									
223.	77	EB	C	1-5 years	Neg	0									
224.	77	EB	C	1-5 years	Neg	0									
225.	**78**	**NIB**	**A**	**3-4 months**	**Pos**	**12**									
226.	**78**	**NIB**	**A**	**3-4 months**	**Pos**	**10**									
227.	78	NIB	A	3-4 months	Neg	0									
228.	79	NIB	F	?	Neg	0									
229.	80	EB	C	3-4 months	Neg	0									
230.	80	EB	C	3-4 months	Neg	0									

Appendix 2: List of Abbreviations

1. B-A: Bland-Altmann
2. CRI: Continuous Rate Infusion
3. EB: Eastern Brown
4. EPG: Eggs Per Gram
5. GIT: Gastro-Intestinal Tract
6. LOA: Limits Of Agreement
7. $MgSO_4$: Magnesium sulphate
8. NA: Not Available
9. Neg: Negative
10. NIB: North Island Brown
11. NZ: New Zealand
12. ONE: Operation Nest Egg
13. OPG: Oocysts Per Gram
14. PAPP: para-aminoproriophenone
15. PF2050: Predator Free New Zealand2020
16. Pos: Positive
17. Rpm: revolutions per minute
18. RR: Reference Range
19. se: standard error
20. SoVS: School of Veterinary Science
21. spg: Specific gravity
22. VNK: Ventricular nematodiasis in kiwi
23. $ZnSO_4$: Zinc sulphate

131

132