A STUDY OF COCCIDIAL PARASITES

IN THE HIHI (*NOTIOMYSTIS CINCTA)*

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE

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ABSTRACT

A systemic protozoal disease resembling atoxoplasmosis has been found to be a serious problem in the captive hihi population at the National Wildlife Centre (N.W.C.), Mt Bruce, Masterton, causing high juvenile mortality. The literature on the Genus *Atoxoplasma* is reviewed, with attention focusing on the taxonomy, history, and life cycle of the organism, named and unnamed species, identification, epidemiology and clinical signs of infection. *Atoxoplasma*-like organisms have been recognized in birds since 1900 but difficulties in identification and in classification have meant that the genus is still inadequately defined and poorly understood.

Monitoring of oocyst shedding from captive hihi at the N.W.C. during the 1997-1998 and 1998-1999 breeding seasons confirmed that the most consistent shedding was by the chicks/juveniles which had at least two periods of shedding: one in the nestling stage and one post-fledging. The earliest recorded excretion was at 9 days of age. Post-fledging, there was a period of high oocyst shedding between 6.5-8 weeks of age during both seasons. Some chicks had intermittent periods of excretion of high numbers of oocysts throughout the year although the months of December through to, and including, February were the times when high numbers of oocysts were shed by the chicks most consistently.

The adult hihi at the N.W.C. passed oocysts only sporadically, with the exception of one hand-reared bird which had little exposure to conspecifics as a juvenile, and another bird that was in poor health at the time of shedding. Small numbers of coccidial oocysts were also present in faeces collected from hihi on Tiritiri Matangi and Mokoia Islands but, largely because of infrequent sampling, no shedding patterns were discernible. It is proposed that hihi normally develop immunity to this coccidial organism as they mature if they are reared naturally, but might shed oocysts if suffering from concurrent disease.

Treatment with toltrazuril (Baycox solution 2.5%, Bayer) eliminated the shedding of oocysts in all birds. However, oocyst numbers sometimes rose again very quickly

suggesting that toltrazuril is effective against the intestinal forms of this coccidia but not against the extra-intestinal forms.

Difficulties were experienced in the *in vitro* sporulation of oocysts shed by birds from the N.W.C. although those recovered from the two islands sporulated relatively easily. The reasons for this were not established but it is suggested that the sporulation difficulties may have been due to management factors at the captive institution, such as the use of some medications. Preliminary morphological characteristics of sporulated oocysts of the *Isospora*-type are described. Two main types of coccidia were identified: Group A which comprised coccidia which had subspherical oocysts, and Group B which had ellipsoidal oocysts. Both types of coccidia were found in birds from all three locations.

These preliminary epidemiological studies suggest that infection is maintained in chicks and juveniles with oocysts remaining viable in the environment for extended periods of time. Further work on oocyst shedding by adults during the breeding and oocysts viability in the environment is required in order to confirm this hypothesis.

Transmission studies using starlings as recipient birds for both starling and hihi oocysts were not completed because of the unavailability of appropriate infective material at the required time. Another study using a single hihi as the recipient of sporulated hihi oocysts was also not completed because of the death of the hihi due to a fungal infection. A transmission study where sporulated hihi oocysts were inoculated into zebra finches, was completed and there was no evidence of infection, supporting the belief that these coccidia are species-specific.

The gross and histological findings on necropsy of 12 cases of coccidial infection in hihi from the N.W.C. are described in detail including the locations of the various coccidial forms within the body. These findings are compared with cases of *Atoxoplasma* and *Atoxoplasma*-like infections in birds recorded in the literature. The most outstanding feature of the infection in hihi is the intestinal pathology which involves extreme

thickening of the lamina propria with an overwhelming invasion by coccidial forms into the lamina propria and the intestinal epithelial cells. No atoxoplasmosis cases in other avian species exhibit similar intestinal pathology. Although there are some common aspects in the hepatic and splenic pathology, and in the tissue location of the different coccidial life cycle stages, there is currently insufficient consistent similarity to justify placing the hihi coccidia in the Genus *Atoxoplasma.* The taxonomic classification of this coccidia therefore remains uncertain.

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The hihi (*Notiomystis cincta*), or stitchbird, is an endangered native New Zealand bird from the same family (*Meliphagidae*) as the tui (*Prosthemadera novaeseelandiae)* and the bellbird (*Anthornis melanura*), both of which are relatively common. The only self-sustaining population of the hihi is on Little Barrier Island. This population is relatively small and might only comprise 2000 individuals (Castro, 2000). A series of translocations to off-shore islands has been carried out by the Department of Conservation (DoC) since 1980, in order to reduce the likelihood of a catastrophe eliminating the species altogether. A captive breeding population has been established at the National Wildlife Centre (N.W.C.), Mt Bruce, Wairarapa, and this is used to learn more about the species, their life history, their management, and health issues. The population is also invaluable for bringing the species to the public's attention as there are no other places on the mainland of New Zealand where the hihi can be seen. Surplus birds from this captive population are transferred to islands, particularly Tiritiri Matangi Island in the Hauraki Gulf.

There has been a high rate of hihi juvenile mortality at the N.W.C. and the main cause of death, up until and including the 1998-1999 season, has been a coccidial infection. It has been suggested that this infection is caused by an *Atoxoplasma*-like organism (Stockdale and Charleston, pers. comm.) because of the finding of coccidial forms in extra-intestinal tissues such as liver and spleen.

It was deemed necessary to further investigate this syndrome in order to try to identify the organism involved, elucidate its life cycle, study and document the resulting pathology,

and determine factors associated with the transmission and acquisition of infection. It was hoped, therefore, that this information would help in the successful management of the bird and specifically reduce mortality due to the disease.

It was suggested by Doré in 1918 that it may be possible to connect the comparatively rapid disappearance of New Zealand native birds with the introduction of exotic protozoa through the medium of the imported fauna. Myers (1923) elaborated further on this belief stating that the effects of an introduced avian disease is perhaps the only explanation which could even partially explain the wholesale disappearance of certain species from untouched areas in New Zealand before the effects of introduced mammals or other causative factors. This theory was also supported by Stockdale (pers. comm.) in relation to the hihi but it seems likely that too long a period of time has now lapsed for this to be completely proven or disproven.

1.2 THE GENUS *ATOXOPLASMA*

1.2.1 Taxonomy

The protozoa are single-celled, eukaryotic organisms which form a sub-kingdom of the kingdom Protista. Within the Protista, the phylum Apicomplexa contains many important parasitic organisms, in fact all the members of the Apicomplexa are parasitic (Levine, 1988). Their name is derived from the presence of an apical complex which each member has at some stage in their development and which is used to attach to, or gain entry into host cells. Apicomplexans are often banana-shaped and locomotion is through undulations effected by internal organelles (Hendrix, 1998).

The phylum Apicomplexa is divided into the classes *Aconoidasida, Perkinsasida* and *Conoidasida* (Levine, 1988). The class *Aconoidasida* includes the haemosporids which multiply in erythrocytes, are transmitted by blood-sucking insects and include the malaria parasites, and the piroplasms which also multiply in erythrocytes but are transmitted by ticks. The class *Perkinsasida* contains parasites of invertebrates e.g. *Perkinsus marinus* which is found in the American oyster (Levine, 1988). The members of the class *Conoidasida* generally produce oocysts in their life cycles which contain infective sporozoites after sporogony. Within this latter class is the subclass *Gregarinasina*, the members of which are found in invertebrates or lower chordates. The other main group within this class is the subclass *Coccidiasina*, the members of which Levine (1988) refers to as coccidia. It is within this subclass that we find most of the Apicomplexa of veterinary interest. Within this subclass is the order *Eucoccidiorida* which contains the suborder *Adeleorina* (containing the haemogregarines which live in blood cells of fish, reptiles and other vertebrates) and the suborder *Eimeriorina*, the group commonly referred to by many authors as the coccidia.

The suborder *Eimeriorina* contains many families including the *Eimeriidae* (which includes the genera *Eimeria* and *Isospora*), *Lankesterellidae, Sarcocystidae* (the members of which are unusual in that their life cycles involve asexual stages in a prey animal and sexual stages in a predator animal), and the family *Atoxoplasmatidae.* The members of the family *Atoxoplasmatidae* are homoxenous with merogony in the blood and intestinal cells, gametogony in intestinal cells of the same individual host, sporogony outside the host, and transmission by ingestion of sporulated oocysts (Levine, 1982).

1.2.2 History

Garnham (1950) described a parasite in monocytes and lymphocytes of a chatterer, *Argya rubiginosa,* and a shrike, *Lanius collaris,* in East Africa. Similar parasites in birds, which had formerly been called avian *Toxoplasma,* had been previously described by Laveran (1900), Adie (1908), Aragão (1911, 1933) and Marullaz (1913). Garnham (1950) named the parasites *Atoxoplasma* and defined them as "parasites which inhabit the monocytes of birds from many parts of the world, are strictly host specific, non-pathogenic and possess a delicately granular cytoplasm not enclosed by a periplast, and a large diffuse nucleus with a tiny karyosome".

Lainson (1959) reported on a study of *Atoxoplasma* in English house sparrows (*Passer domesticus domesticus)* and described the life-cycle of the organism in these birds. Young birds were found to be infected while still in the nests as early as 6 days after hatching. All stages of schizogony were found in the lymphoid-macrophage cells of the spleen, bone marrow and liver of the birds, and later, gametocytes developed in similar cells of the liver, lungs and kidney of the same species. Dissanaike (1967) also reported sexual stages of this parasite in visceral tissues, this time in mynah birds (*Acridotheres tristis melanosternus*). Lainson (1959) suggested that the transmission of the parasite in sparrows took place via ingestion of infected mites (*Dermanyssus gallinae),* however, Box (1967) found no effect

on *Lankesterella* patency from association with, or oral administration of mites (*Ornithonyssus bursa*). After assessing the differences between the known species of *Lankesterella* and *Atoxoplasma,* Lainson could find no reason why they should be separated as two distinct genera and proposed that the name *Atoxoplasma* Garnham, 1950, be regarded as a synonym for *Lankesterella* Labbé, 1899.

Box (1967) reported that administration of *Isospora* oocysts to sparrows and canaries (*Serinus canarius*) was associated with an increased rate of parasitaemia and death from *Lankesterella*. In further studies Box (1970, 1977) reported that the parasites of mononuclear cells found in visceral tissues of canaries resembling and previously classified as *Atoxoplasma,* were, in fact, asexual stages of *Isospora* and proposed the name *Isospora serini* for this parasite. She found the sexual stages were present in intestinal epithelium. Box (1977) also described the life-cycle of *I. canaria* in the canary. This parasite has a conventional coccidian life-cycle in that all the endogenous stages are in the epithelium of the small intestine.

Levine (1982) recommended "that the generic name *Atoxoplasma* be retained and that it should be placed in a new family, *Atoxoplasmatidae,* containing homoxenous coccidia with merogony in the blood and intestinal cells, gametogony in the intestinal cells of the same host individual, and sporulation outside the host". He formulated a list of 19 species of *Atoxoplasma* and noted that the complete life-cycles of most of these species had not been determined. These species are listed in Table 1.

Cooper *et al.* (1989) and Martinez and Muñoz (1998) regarded atoxoplasms as tissue-invading forms of the coccidian *Isospora.* This is in contrast to McNamee *et al. (*1995*)* and Quiroga *et al.* (2000) who considered that *Atoxoplasma* were merely related to members of the genus *Isospora* in which merogony and gametogony are restricted to intestinal epithelial cells with no development phase in the cells of the lymphoid-

macrophage system. At the present time, therefore, the exact nature of atoxoplasms and how they behave, has not been conclusively established.

1.2.3 Life Cycle

Coccidia occur in all vertebrates that have been examined for them and also in a few invertebrates (Levine, 1988). Most host species have a number of coccidia species and it is generally held that the majority of coccidia are host-specific. Most coccidia are parasites of intestinal cells and they are a common and important cause of enteric disease in many domestic mammals and birds.

The life cycle of the members of the *Coccidiasina* (from here on referred to as the coccidia) follows the basic apicomplexa life cycle with modifications within different groups and with some types of coccidia lacking certain stages (Levine, 1988). Reproduction alternates between sexual and asexual phases. The basic pattern is that a zygote is formed by the fusion of gametes (syngamy). The zygote or sporont (which is generally within an oocyst wall) then divides by the process of sporogony to form sporozoites which may lie in sporocysts or be free in the oocyst (Current *et al.*, 1990). In most coccidia species, sporulation occurs only outside the host but there are exceptions (e.g. *Sarcocystis* and *Frenkelia*) where sporulation takes place inside the host's body. The resulting sporulated oocyst is the infective stage for the host. The excreted oocysts are ingested by the host, excystation occurs by which the sporozoites become free from the oocyst (or in the case of *Sarcocystis,* from the sporocysts), and then these invade intestinal epithelial cells. Once inside the host cells the sporozoites round up and become a uninucleate meront/schizont/trophozoite (Current *et al.*, 1990). These divide asexually by multiple fission (merogony/schizogony) to form merozoites/schizozoites which leave the host cells and either continue on with further cycles of merogony or enter into sexual reproduction (gametogony). In gametogony, the merozoites enter host cells and become

microgamonts (male gamonts) or macrogamonts (female gamonts). The microgamonts undergo repeated nuclear division followed by cytoplasmic divisions resulting in several microgametes which eventually fertilize macrogametes producing the zygote. There are therefore three multiplications in the basic apicomplexan life cycle: sporogony, merogony and gametogony.

The life cycles of most *Atoxoplasma* species are unknown and precisely which species can be assigned to this genus is unclear. The life cycle of the atoxoplasms is believed to follow the basic pattern of the coccidial life cycle, with the addition of extra-intestinal stages which occur after sporozoites released in the intestinal lumen invade lymphocytes and macrophages as well as epithelial cells (Partington *et al.*, 1989). The sporozoites are then able to be disseminated throughout the tissues of the body. The life cycle involves the production of an isosporan oocyst, which is composed of two sporocysts, each containing four sporozoites. Sporulation occurs outside the host and infection is considered to be direct via the faecal-oral route.

Levine's (1982) definition of the family *Atoxoplasmatidae* as containing homoxenous coccidia with merogony in blood and intestinal cells, gametogony in the intestinal cells of the same host individual, and sporulation outside the host, has been widely used and can still be used in defining *Atoxoplasma* today. However, the distinction between this family and other related organisms such as *Isospora*, appears to be no longer so definite nor universally agreed upon. This is in part because of the finding of extraintestinal stages of feline and canine *Isospora* species in the early 1970s (Dubey and Frenkel, 1972; Dubey, 1975, 1978, 1979). Box (1975) found asexual stages of *Isospora serini* in extraintestinal mononuclear phagocytes in the canary and Milde (1979) reported that extraintestinal coccidial stages also occurred in sparrows and that these stages belonged to the life cycle of stages appearing in the intestine. Taking these findings into account, Levine (1982) later reclassified *Isospora serini* as *Atoxoplasma serini.*

Rossi *et al.* (1996) found schizont-like elements in the liver, spleen, lungs, heart and pancreas of black siskins (*Carduelis atrata*, a native South American finch) infected with *Isospora atrata*. There have been reports of disseminated extraintestinal isosporiasis in human patients with AIDS who are infected with *Isospora belli* (Restrepo *et al.*, 1987; Michiels *et al.*, 1994). It is thought that these extraintestinal tissue cysts are probably a reservoir of developmental stages that can recolonize the intestine and cause recrudescence of clinical disease (Lindsay *et al.*, 1997). This is very similar to what occurs with *Atoxoplasma*, where extraintestinal stages (tissue cysts are not produced in this genus) most probably account for both the ability of the infection to become apparent and/or clinical after a latent period due to the parasites returning to the intestine to initiate further infections (Milde, 1979), and the chronicity of infection (Box, 1977), without the possibility of re-infection. This latter feature can be accounted for by the fact that the parasites are present in mononuclear cells extra-intestinally, and these are long-lived cells compared to intestinal epithelial cells which have a life span of two-three days at most (Box, 1977).

1.2.4 Species of *Atoxoplasma*

Levine (1982) devised a list of named *Atoxoplasma* species (see Table 1). Levine (1982) states that it is possible that some of the forms which he has assigned to *Atoxoplasma* may eventually be found to belong to other genera, and, in addition, there are probably "*Isospora*" species that are likely *Atoxoplasma*. Baker *et al.* (1972) recorded many atoxoplasms which Levine did not include because they had not been described adequately. More recently, *Atoxoplasma* species have been recorded from further species, including: the nutmeg mannikin (*Lonchura punctulata*), house finch (*Carpodacus mexicanus*) and Japanese white-eye (*Zosterops japonica)*(van Riper *et al.*, 1987), the Bali mynah (*Leucopsar rothschildi*) (Partington *et al.*, 1989; Norton *et al.*, 1995), the greenfinch (*Carduelis chloris*) (Cooper *et al.*, *1989*; Ball *et al.*, 1998), the bullfinch (*Pyrrhula pyrrhula*) (McNamee *et al.*, 1995), the black siskin (Rossi *et al.*, 1997), a

hybrid passerine (*Serinus canarius* X *Carduelis* cannabina) (Martinez and Muñoz, 1998), and the Indochinese white-rumped shama (*Copsychus malabaricus interpositus*) (Harvey *et al.*, 1997). These species have not yet been formally classified.

Table 1.1 : Species of *Atoxoplasma* (based on Levine, 1982)

Species	Synonyms	Host species
1. *A. adiei* (Aragao, 1911) Baker, Bennett, Clark, and Laird, 1972	*Haemogregarina adiei* Aragao, 1911; *Lankesterella adiei* (Aragao, 1911) Lainson, 1959; *L. passeris* Raffaele, 1938; *L. garnhami* Lainson, 1959; *Toxoplasma passeris* Rousselot, 1953.	House sparrow *Passer domesticus* (L.)
2. *A. amadinae* (Fantham, 1924) Baker, Bennett, Clark, and Laird, 1972	*Leucocytogregarina amadina* Fantham, 1924: *Hepatozoon amadinae* (Fantham, 1924) Wenyon, 1926.	Red-headed weaver finch *Amadina erythrocephala* (L.).
3. *A. argyae* Garnham, 1950	*Lankesterella argyae* (Garnham, 1950) Lainson, 1959.	Chatterer *Turdoides* (syn. *Argya*) *rubiginosus* (Ruppell).
4. *A. avium* (Labbe, 1894) Baker, Bennett, Clark, and Laird, 1972	*Drepanidium avium* Labbe, 1894; *Lankesterella avium* (Labbe, 1894) Labbe, 1899.	Northern shrike *Lanius excubitor* L., magpie *Pica pica* (L.), raven *Corvus*

		corax L., buzzard *Buteo buteo* (L.), kestrel *Falco tinnunculus* L., owl *Asio* (syn., *Strix*) *flammea* (Pontoppidae), and owl *Strix* (syn., *Syrnium*) *aluco* L. (modern research is needed to determine the true host[s]).
5. *A. butasturis* (de Mello, 1935) nov. comb.	Syn., *Toxoplasma butasturis* de Mello, 1935	*Butastur teesa* (Franklin).
6. *A. coccothraustis* Corradetti and Scanga, 1963.		Finch *Coccothraustes coccothraustes* (L.).
7. *A. corvi* (Baker, Lainson, and Killick-Kendrick, 1956) nov. comb.	*Lankesterella corvi* Baker, Lainson, and Killick-Kendrick, 1956.	English rook *Corvus f. frugilegus* L.
8. *A. danilewskii* Zasukhin, Vasina, and Levitanskaya, 1957		finch *Carduelis* (syn., *Spinus*) *spinus* (L.).
9. *A. desseri* n.sp.	*Lankesterella* sp. Khan and Desser, 1971; *Isospora* sp. (Khan and Desser, 1971) Desser, 1980.	Evening grosbeak *Coccothraustes* (syn., *Hesperiphona*) *vespertinus* (Cooper) and rose-breasted grosbeak *Pheucticus ludovicianus* (L.)

10. *A. lainsoni* (Dissanaike, 1967) comb. nov.	*Lankesterella lainsoni* Dissanaike, 1967.	Ceylon mynah bird *Acridotheres tristis melanosternus* Legge.
11. *A. liothricis* (Laveran and Marullaz, 1914) Baker, Bennett, Clark, and Laird, 1972.	*Toxoplasma liothricis* Laveran and Marullaz, 1914.	Japanese babbler *Leiothrix lutea* (Scopoli).
12. *A. paddae* (Aragao, 1911) Laird, 1959 (TYPE SPECIES).	*Haemogregarina paddae* Aragao, 1911; *Toxoplasma avium* Marullaz, 1913; *Atoxoplasma avium* (Marullaz, 1913) Garnham, 1950; *Lankesterella paddae* (Aragao, 1911) Lainson, 1959.	Java sparrow *Padda oryzivora* (L.), possibly silvereyes *Zosterops lateralis* (Latham), *Z. flavifrons* (Gmelin), *Z. rennelliana* Murphy, and *Woodfordia superciliosa* North, and improbably Stewart Island weka (rail) *Gallirallus a. australis* (Spaarman) (modern research is needed to determine the true host[s]).
13. *A. paulasousai* (Correa, 1928) nov. comb.	*Haemogregarina paulasousai* Correa, 1928.	Brazilian tanager *Stephanophorus diamedatus* (Temminck).
14. *A. pessoai* (Correa, 1928) nov. comb.	*Haemogregarina pessoai* Correa, 1928.	Brazilian sparrow *Poospiza thoracica* (Nordman).
15. *A. picumni* (Mackerras	*Lankesterella picumni*	Australian treecreeper

and Mackerras, 1960) nov. comb.	Mackerras and Mackerras, 1960.	*Climacteris picumnus* Temminck and Laugier.
16. *A. serini* (Aragao, 1933) nov. comb.	*Haemogregarina serini* Aragao, 1933; *Lankesterella serini* Lainson, 1959; *Isospora serini* (Aragao, 1933) Box, 1975.	Canary *Serinus canaria* (L.)
17. *A. sicalidis* (Aragao, 1911) Baker, Bennett, Clark, and Laird, 1972.	*Haemogregarina sicalidis* Aragao, 1911; *Hepatozoon sicalidis* (Aragao, 1911) Hoare, 1924.	Brazilian canary *Sicalis flaveola* (L.).
18. *A. spermesti* (Rousselot, 1953) nov. comb.	*Hepatozoon spermesti* Rousselot, 1953; *Lankesterella spermesti* (Rousselot, 1953) Box 1964; *Toxoplasma* sp. Wenyon, 1926 from *Spermestes cucullatus.*	Munis *Lonchura (*syn. *Spermestes*) *c.cucullatus* (Swainson).
19. *A. sporophilae* (Aragao, 1911) Baker, Bennett, Clark, and Laird, 1972.	*Haemogregarina sporophilae* Aragao, 1911; *Hepatozoon sporophilae* (Aragao, 1911) Hoare, 1924.	Sparrow *Sporophila albogularis* (Spix).
20. (unnamed) van Riper, van Riper and Laird, 1987		nutmeg mannikin *Lonchura punctulata*

21. (unnamed) van Riper, van Riper and Laird, 1987		house finch *Carpodacus mexicanus*
22. (unnamed) van Riper, van Riper and Laird, 1987		Japanese white-eye *Zosterops japonica*
23. (unnamed) Partington, Gardiner, Fritz, Phillips, Montali, 1989		Bali mynah *Leucopsar rothschildi*
24. (unnamed) Cooper, Gschmeissner and Greenwood, 1989		greenfinch *Carduelis chloris*
25. (unnamed) McNamee, Pennycott and McConnell, 1995		bullfinch *Pyrrhula pyrrhula*
26. (unnamed) Rossi, Perrucci, Taccini, Vitali, Braca and Renzoni, 1997		black siskin *Carduelis atrata*
27. (unnamed) Harvey, Rideout, Papendick, Sutherland-Smith, Stalis and Gardiner, 1997		Indochinese white-rumped shama (*Copsychus malabaricus interpositus*)
28. (unnamed) Martinez and Muñoz, 1998		hybrid passerine *Serinus canarius* X *Carduelis cannabina*

In New Zealand, Laird (1950) recorded the finding of *Toxoplasma* organisms (subsequently assigned to the genus *Atoxoplasma*) in mononuclear cells in blood smears from a silvereye (*Zosterops lateralis*), a species which reached New Zealand apparently from Australia in 1856 (Oliver, 1930). Although this is the first record of *Atoxoplasma* from New Zealand, it is possibly not the first record in silvereyes as Rosenbusch (1932) recorded an infection of "toxoplasmosis" in a similar species (*Zosterops palpebrosar peguensi*) in Argentina. In 1959, Laird recorded the finding of *Atoxoplasma* organisms in mononuclear cells in blood smears from a chick of the Stewart Island Weka (*Gallirallus australis scotti*), captured in December, 1950. This appears to be the first record of *Atoxoplasma* in a rail and is the first record of an *Atoxoplasma* infection in an indigenous New Zealand bird. Levine (1982) postulated, without substantiation, that the parasite found in the weka was not likely to be *Atoxoplasma* while that found in the silvereye was.

1.2.5 Identification

Sporulated oocysts of *Atoxoplasma* and *Isospora* have similar morphology since both contain two sporocysts with four sporozoites in each. This makes diagnosis by faecal examination difficult. In addition, faecal shedding of oocysts from an infected bird can be intermittent. Mesher and Mauldin (1996) claim that these two genera of oocysts, when shed from canaries, can be differentiated on the basis of their oocyst size (Box, 1975). This is presumably based on the premise that canaries commonly shed only one species of *Isospora* (i.e. *Isospora canaria*). The coccidial fauna of most other avian host species is not so well-defined and most probably have more that one species. Another factor relating to the difficulty in identifying coccidial species on oocyst morphology, is that the length, width and shape of oocysts may change during infection and as a function of the inoculum, according to Cheissin (1947, 1957) as cited by Svobodová (1994). Furthermore, oocyst and sporocyst size might vary amongst host individuals of one species, or among different species of one genus (Gardner and Duszynski, 1990). However, the morphology of oocysts continues to be very important in identification.

It may be possible to diagnose *Atoxoplasma* infections ante-mortem by the demonstration of characteristic intracytoplasmic zoite forms in lymphocytes or monocytes in blood smears (Box, 1966; Khan and Desser, 1971; Baker *et al.*, 1972; Mesher and Mauldin, 1996). However, the use of blood smears as the only means of diagnosis is not always reliable because the presence of parasitaemia in mononuclear cells of peripheral blood is inconstant (Quiroga *et al.*, 2000). One of the basic factors underlying the controversy surrounding the classification of *Atoxoplasma,* is that there are several different genera of protozoa that have stages in blood cells that look similar (Levine, 1982). These cannot correctly be assigned to their proper genera without knowing their life-cycles, studies of which are difficult to carry out in the laboratory. Most species of *Atoxoplasma* have been described exclusively from examination of blood films or tissue smears (Ball *et al.*, 1998). In earlier years these tissue stages were not thought to be associated with faecal oocysts.

Partington *et al.* (1989) suggest that the most accurate method for diagnosing *Atoxoplasma* infection ante-mortem may be with Wright's-stained impression smears from liver biopsies, provided the bird can stand the stress of the procedure. Partington *et al.* (1989) also state that the best method for diagnosing the infection post-mortem is by impression smears of the liver and spleen. Neither examination of blood smears nor liver biopsies was a major part of the present author's research and so these methods of identification will not be dealt with in any detail.

Quiroga *et al.* (2000) recommend ultrastructural examination of the coccidial organisms in tissues for the diagnosis of atoxoplasmosis, along with light microscopy. Ball *et al.* (1998) studied the ultrastructure of coccidial asexual and sexual stages in the tissues of greenfinches and found the structures to be consistent with *Atoxoplasma* organisms described from other bird species e.g. by Desser (1980).

1.2.6 Epidemiology and clinical signs

The clinical syndrome of coccidiosis is largely a disease of domestication, occurring when the host species is crowded and confined together, enabling the buildup of large numbers

of oocysts in the environment (Fayer, 1980). For this reason coccidia are believed to be generally harmless in the wild (Levine, 1974).

Factors which affect the number of oocysts produced by an animal, include: the inherent reproductive potential of each species in a non-immune host, the immunity of the host, the "crowding" factor where as more oocysts are inoculated, the number of oocysts produced per oocyst inoculated decreases, the nutritional status of the host, the strain of the host, the strain of the parasite, and various stress factors such as exposure to climatic changes and changes in diet (Fayer, 1980). Immunity is specific to each coccidian species, and immunity to one species does not confer immunity to other species in the same host. It has been found that certain strains within an animal species are differentially resistant to coccidiosis (Lillehoj and Trout, 1993). Natural infection by coccidia is generally thought to induce long-lasting immunity (Lillehoj and Trout, 1993).

The primary mode of transmission of *Atoxoplasmosis* is via the faecal-oral route (Flammer *et al.*, 1989) by ingestion of sporulated oocysts. Factors affecting sporulation (temperature, moisture and aerobia), oocyst survival time, and oocyst dispersal, all affect the epidemiology of coccidial infections (Fayer, 1980).

The occurrence of the disease has been associated with poor aviary hygiene (McNamee *et al.*, 1995), overcrowding, and inadequate nutrition (Mesher and Mauldin, 1996). Consequently, in order to limit infection, it has been recommended by McNamee *et al.* (1995), that a high standard of aviary hygiene be maintained and that drinking water and bathing bowls are frequently replaced to prevent ingestion of the sporulated oocysts. It has been suggested that treatment does not always result in a cure although oocyst shedding may be diminished (Partington *et al.*, 1989).

Monoxenous coccidia are thought to be genus-specific (Levine, 1982), although there are exceptions e.g. *Isospora xerophila* (a parasite of birds) infects four genera of the family Ploceidae (Barré and Troncy, 1974) . Garnham (1950) considered *Atoxoplasma* to be host specific and this is supported by the work of Box (1981) and Khan and Desser (1971).

The house sparrow is the most commonly reported host for *Atoxoplasma* (van Riper *et al.*, 1987). Prevalences have been found to be as high as 100% in some sparrow populations (Lainson, 1959) and as low as 2.9% in a survey in North America (Greiner *et al.*, 1975).

Both sub-clinical (Lainson, 1959) and clinical infections have been recorded, from many parts of the world including the U.K., Europe, U.S.A., and Indonesia. *Atoxoplasma* has been associated with clinical disease in canaries (Box, 1977), sparrows (Lainson, 1950), greenfinches (Cooper *et al.*, 1989), Bali mynahs (Partington *et al.*, 1989), bullfinches (McNamee *et al.*, 1995), and Indochinese white rumped shama (Harvey *et al.*, 1997). Signs of disease include diarrhoea, weight loss, ruffled feathers, distended abdomen, dehydration, pectoral muscle atrophy, pale mucous membranes, and acute death. In some cases there had been a decreased appetite (Cooper *et al.*, 1989) and in others, the birds have had an abnormally increased appetite (Rossi *et al.*, 1996). In the many of the reported clinical cases the disease affected young birds and resulted in a high mortality. In the case of the greenfinches (Cooper *et al.*, 1989), where the infection was associated with the syndrome known as "going light", the birds were affected at weaning and there was usually a high mortality. In the Bali mynah however, the clinical history of affected birds usually consists of acute death in fledging birds, most often between 3 and 8 weeks of age (Norton *et al.*, 1995).

CHAPTER TWO

PARASITOLOGY

2.1 INTRODUCTION

For several years hihi at the National Wildlife Centre (N.W.C.), Mount Bruce, Masterton, have been known to shed *Isospora*-like oocysts in their faeces. Sampling before 1997 had shown that oocysts were present in the faeces of adults and juveniles at various times from October to February (Helen Gummer, unpublished breeding season report 1997) with shedding times apparently coinciding at times with nest-building, copulation, incubation, nestling and fledging. Coccidiosis was identified as the main cause of juvenile hihi mortality at the N.W.C., affecting hihi up to the age of approximately 2 months (Stockdale, Alley, Christensen, pers. comm.). Juvenile mortality was approximately 50% (Helen Gummer, unpublished breeding season report 1997) and was having a large detrimental effect on the successful rearing of this species in captivity. Control of the infection was not being achieved. Neither the parasites, nor disease arising from these parasites, had been recorded from hihi in the wild.

By far the bulk of parasitological investigation of avian coccidia has focused on poultry and therefore on *Eimeria* species. However, Box (1970, 1975, 1977) made a major contribution to the field with her research on *Isospora* (and *Atoxoplasma*) infections in sparrows (*Passer domesticus domesticus)* and canaries (*Serinus canarius)*, work which involved faecal examinations for the detection of the oocysts. Many other researchers have concentrated primarily on the blood stages of *Atoxoplasma*-like organisms e.g. Laird (1950, 1959), and van Riper *et al.*(1987) with little investigation of the oocysts. Boughton (1937) produced some early work in this area with his studies on oocyst production in avian coccidiosis in passerines. He described a method for counting oocysts in dried faecal samples from small birds and also looked in detail at characteristics of oocyst production (such as diurnal periodicity of shedding in sparrows) and characteristics of

Isospora infections in sparrows. In recent years, there have been more studies on *Isospora*-type infections in free-living birds from various areas of the world, where oocyst examinations have been employed e.g. Svobodová (1994), Poon and Chew (1991).

Examination of blood samples has been used as an important means of identifying protozoal species which have blood stages, such as *Atoxoplasma* (Box, 1966; Khan and Desser, 1971; Baker *et al.*, 1972; Mesher and Mauldin, 1996). However, blood collection from affected birds was not possible in this investigation because the vulnerable conservation status of the species necessitated minimal handling.

The present investigation was undertaken to determine, in more detail, how oocyst excretion varied over time in the different age groups of birds in the captive hihi population at the N.W.C. From this it was hoped to be able to identify/confirm the disease risk periods in relation to the time of year and the age of the bird, as well as identify the influence of activities such as nest-building, copulation, drug treatments, and any unknown factors on shedding. Because the morphological characteristics of the oocyst are important in the classification of coccidia (Box, 1975; Long and Joyner, 1984), a successful and suitable sporulation technique also needed to be found.

2.2 MATERIALS AND METHODS

2.2.1 Collection of samples

(i)Captive birds

The majority of faecal samples were collected from the captive hihi population at the N.W.C. The Centre is surrounded by a remnant of native bush, containing at least 8 native avian species, and farmland, containing many common introduced species. This is the only captive population of hihi and it has had a history of high juvenile mortality due to coccidial infection, at least as far back as 1994. The number of captive hihi varied between 3 and 7 adults over the monitoring period (January 1998-December 1999). The population was intensively managed by a small team of permanent staff and several seasonal and volunteer workers during the collection period. The N.W.C. staff member with direct responsibility for the hihi was Rose Collen (see Appendix I for details of management of hihi at the N.W.C.).

Sampling was carried out at frequencies which varied according to the time of year, the age of the birds and the results from the immediately preceding samples. From early January 1998, the hihi faecal samples from the N.W.C. were sent to Massey University for flotation counts and sporulation. Over January, February and March 1998, samples were collected almost daily from the juveniles and their dam, and rarely from other adults. The juveniles were sampled four times during April, reducing to one to two times per month from May to September. The adult birds were not sampled in April or May but were sampled at approximately two-weekly intervals in June and July, one-weekly intervals in August, two-weekly again in September and twice-weekly during October. In November and December there was a large variation in frequency of adult sampling, with one adult ("Keith") being sampled almost daily, and others approximately once every two weeks. The 1998-1999 chicks/juveniles were sampled almost daily up until between 3 ½ - 5 months of age (until the end of May). The adults at this time were generally sampled once

every one to two weeks also up until the end of May. After this time the sampling frequency for all birds declined to one sampling per one to two months, slightly more often in October and approximately every week in November and December. In situations when it was not clear from which particular bird the sample arose, the specimen was labelled as "pooled".

Samples were collected most frequently at those times deemed to be high risk periods. This included the entire nestling period and for approximately two-three months after fledging. Other high risk periods included the time around nest-building for the adults, at times of some drug treatments and at times when external factors were suspected to have induced a stress e.g. unaccustomed handling. Samples were also taken with more frequency when previous samples had been positive, in order to monitor the effects of treatment. When new birds were to be introduced to the aviary from a different locality, the new birds were quarantined, faecal sampled and treated if necessary, before being placed in the existing aviaries and/or mixed with resident birds.

Faecal samples from nestlings were easily gathered from the nests. With the adults, the samples were most often collected from night-time roost sites. These gave the "best" adult samples as the quantities were generally moderate, contamination (e.g. from soil, feed and other bird droppings) was low and the liquid content of the samples was low. Collection from these roost sites was not always possible. Newly fledged juveniles posed a particular problem as they often took some time to use the provided roost sites and it was frequently not obvious where they were roosting. In such cases, samples were gathered from wherever they could be found e.g. on leaves and vegetation. This often meant that samples were very small and with a high level of environmental contamination.

Faeces were generally collected into plain vacutainer tubes which had the advantages of being clean, well-sealed and easily and safely transported. These samples were sent to Massey via a same-day-delivery courier and processing was usually carried out that day or over the next two days. On occasion, processing was delayed longer. During the first 8

months of the project, samples were stored in a refrigerator until transport and processing. Thereafter they were kept at room temperature because of the possibility that refrigerating may inhibit subsequent sporulation.

(ii) Free-living birds

Occasional samples were also obtained from the free-living, but varyingly monitored and managed, hihi population on Mokoia Island, situated in Lake Rotorua.

During collection from Mokoia Island, samples were obtained from nests, and also directly from nestlings. The latter was achieved by holding the nestling in the hand until it defaecated.

2.2.2 Examination of samples

Faecal samples were transferred into a bowl and a small volume (<30ml) of a saturated NaCl solution (specific gravity 1.2) was added. The faeces were ground and stirred through a 660 μm aperture sieve with a spoon until most of the faecal material had been broken up and only a residue remained in the sieve. The sieve and contents were then discarded and the liquid and particulate matter in the bowl was poured into a 30ml glass universal bottle. Further saturated NaCl was used to rinse out the bowl, and the washings added to the bottle. The bottle was filled to the top with NaCl and a meniscus created by adding drops of salt solution. A 22x22mm coverslip (Esco, Biolab Scientific) was placed on this meniscus and a minimum of 10min was allowed for oocysts to float and adhere to the underside of the coverslip and for the coarse material to sink. The coverslip was placed on a glass slide and examined within 30min. The slide had previously been scored into vertical columns of approximately 2mm in width and was examined under a light microscope at 100x magnification and any oocysts counted. *Capillaria* eggs were also frequently seen and counted.

Two main techniques were used to record oocyst numbers: scoring the oocyst quantity and counting the oocysts. Two ways of scoring the oocysts were used: processing all the faeces submitted and scoring the results as "0" to " ++++", or processing all the faeces submitted and presenting the results as 1+ to 5+. These were both based on subjective assessments and were used when the samples were processed by workers other than the author.

Likewise, two counting methods were used: initially the actual number of oocysts present in 1g or less were counted (if less than 1g was submitted, which was generally the case, the oocysts were counted but the faecal weight was not recorded); from 28/8/98, each sample was weighed and the oocysts counted so that the result could be presented as the number of oocysts per gram.

2.2.3 Cleaning and concentrating of oocysts

When oocysts were found in the sample, the following procedure was used to clean and concentrate the oocysts and prepare them for attempted sporulation:

a) All the contents of the universal bottle, apart from the sediment, were emptied into a glass beaker, along with tap water rinsings of the bottle and coverslip.
b) Tap water was added to the beaker to make up to 300ml, which was approximately 10x the volume of the universal bottle contents.
c) The contents were transferred to glass centrifuge tubes.
d) The tubes were centrifuged at 3300xg for 5min.
e) The top ¾ of the supernatant was aspirated and discarded, with a suction pump attached to a tap. Any further oocyst/NaCl mixture remaining was added to the tubes and the suspensions remaining in the tubes were combined after thorough stirring and rinsing with tap water in order to try and retain as many of the oocysts as possible.
f) Steps d) & e) were repeated until only a very small volume of cleaned and concentrated oocysts remained (approximately 2ml or less).

2.2.4 Procedures for attempted sporulation

The following methods were used in an attempt to create favourable conditions for sporulation:

a) After the oocysts had been cleaned and concentrated in tap water, the mixture was placed in a petri dish and 2% potassium dichromate added to give a shallow (<3mm) pool across the bottom of the petri dish. This dish was then placed into a room maintained at 27°C.
b) The same procedure as a) was performed except that 2% sulphuric acid was used instead of 2% potassium dichromate.
c) Both a) and b) were performed as before except that the samples were then held at room temperature.
d) Both a) and b) were performed as before except that the samples were then held in a room at 37°C.
e) After the oocysts had been cleaned and concentrated in tap water, the mixture was placed into a flask and an aquarium oxygen pump added so that air was continuously pumped into the suspension. The mixture was held at 27°C. This process allowed a more efficient turn-over of the surface area to maximize the oocyst exposure to oxygen.
f) All of the above were tried with faeces that had not been exposed to NaCl. This was achieved by using either a portion of a large mass of faeces, the other portion of which had already been floated and found to contain oocysts; or, by using a sample obtained from the same bird subsequent to a sample with a high oocyst count. In both cases, the faeces were mixed through a sieve with tap water. The resulting mixture was concentrated by centrifugation for 5min at 3300xg.
g) A mass of faeces which was known or suspected to contain a high number of oocysts, was maintained as a mass, and placed in a petri dish without a lid which was held inside another larger petri dish containing a small amount of water to maintain 100%

humidity. Both petri dishes were covered by the lid of the larger dish. Faeces prepared this way were maintained at each of the three temperatures mentioned previously.

2.2.5 Assessing sporulation and species identification

The suspensions in petri dishes were checked at regular intervals for evidence of sporulation over a two-three week period. The oocyst suspension was thoroughly stirred and one drop placed on a slide which was then examined at 400x magnification.

Sporulation was assessed as having occurred if sporocysts were present. The numbers of sporulated oocysts per 100 oocysts counted were then recorded.

Measurements were made of sporulated oocysts, using an Olympus BH2 microscope with apochromatic objectives and a digital micrometer.

The following characteristics and measurements of sporulated and partially sporulated oocysts were made and recorded:

a/ The presence or absence of a micropylar cap.
b/ Oocyst size and shape where the shape was classified as round, ellipsoid, ovoid, pyriform, or a combination of any of the four.
c/ Whether the micropyle was distinct or indistinct (if it was present).
d/ Number of polar granules.
e/ Sporocyst size and shape.
f/ The presence or absence of a sporocyst residuum.
g/ The presence or absence of a stieda body.
h/ The number of sporocysts and of sporozoites within them.

2.2.6 Storage of oocysts

Samples containing sporulated oocysts were stored for future use in transmission experiments. The samples were cleaned and concentrated by diluting with sterile distilled water and centrifuging, and then stored in a refrigerator in small capped vacutainers. The caps were removed briefly approximately once every week to ensure adequate oxygen was available to the oocysts.

2.3 RESULTS

2.3.1. Results of faecal examinations of hihi from the National Wildlife Centre

(i) Oocyst shedding from chicks/juveniles in the 1997-1998 breeding season

During January in the 1997-1998 season, large numbers of oocysts were excreted by the chicks before fledging. Oocysts were first found when the chicks were approximately 9 days of age with numbers up to 1650 oocysts per pooled sample by day 20 (Fig 2.1) (Appendix II, Table 1). Oocyst shedding was eliminated with primiquin treatment (primiquin, NZ Defence Force) but numbers quickly built up over a 6-7 day period to previous levels. Likewise, oocyst shedding was eliminated by toltrazuril treatment (Baycox 2.5% solution, Bayer), with oocyst counts at zero by the 5th day of treatment.

Oocyst numbers rose again, although to a lower level, in late February-early March (Fig 2.2) (Appendix II, Table 2) after fledging when the chicks were approximately 8 weeks of age, and responded quickly to toltrazuril treatment (these samples were pooled from all four chicks and the dam). From the 3rd March onwards there was no oocyst excretion from any of the four fledglings for the remainder of 1998 (Appendix II, Table 3).

Fig 2.1 Oocyst shedding by chicks during January 1998

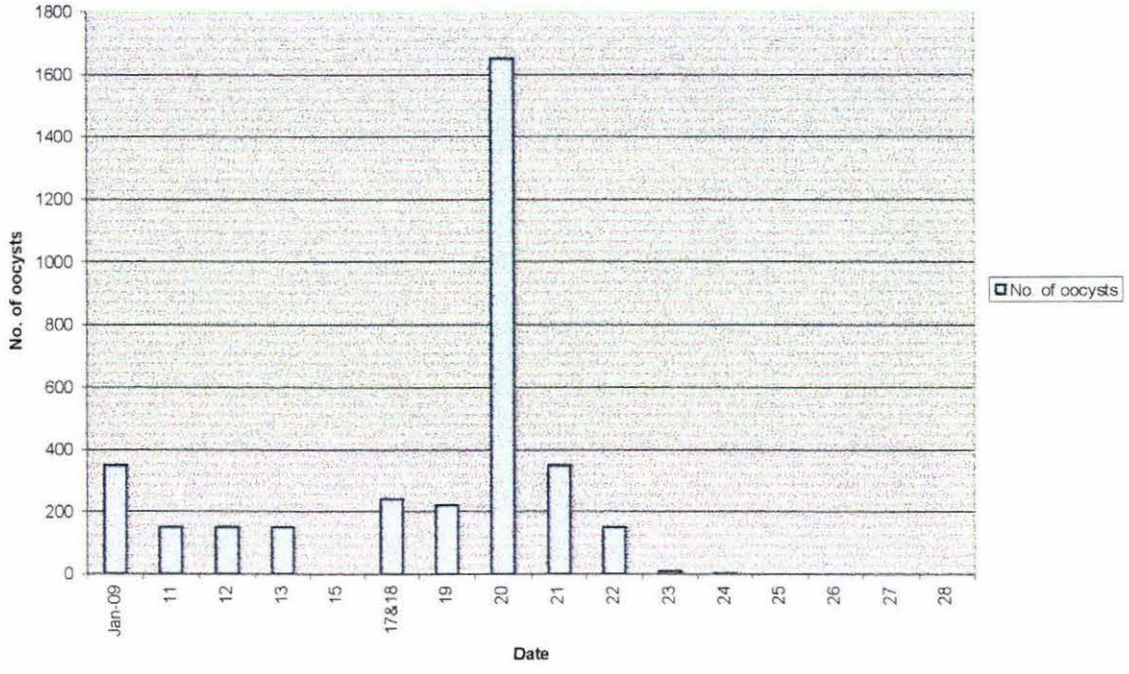
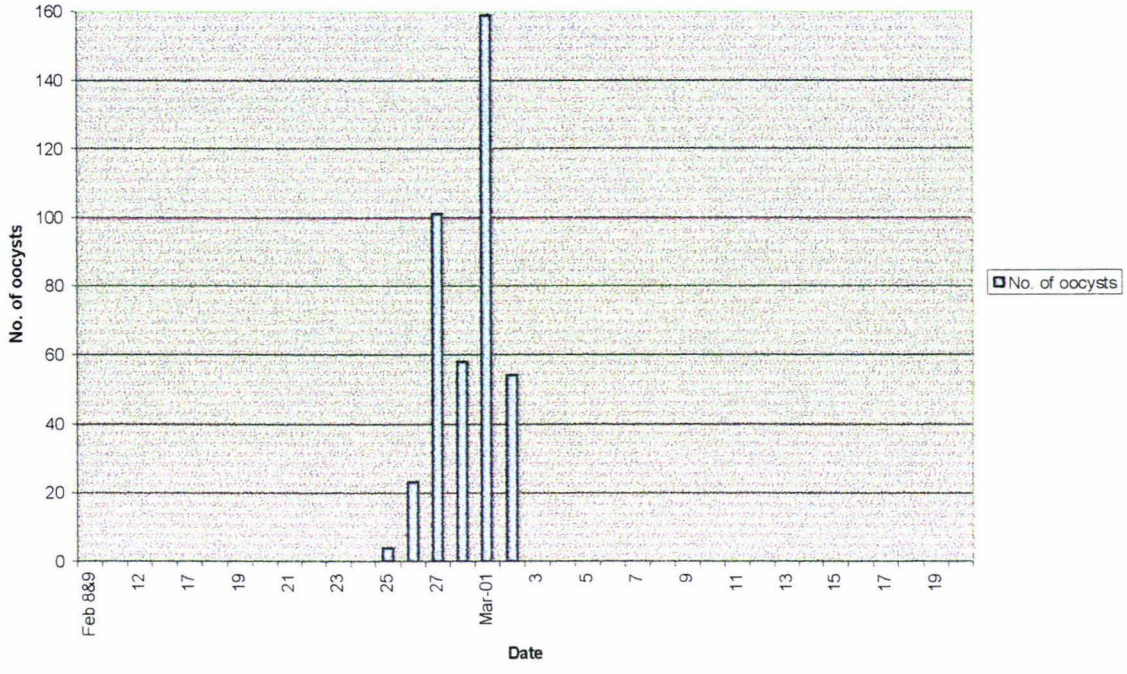


Fig 2.2 Oocyst shedding by juveniles during Feb-March 1998



(ii) Oocyst shedding from adults in the 1997-1998 breeding season

The dam of the chicks, "Jill", excreted a very small number of oocysts in mid-January (Appendix II, Table 4). Although "Jill" is also recorded as having shed moderate numbers of oocysts (up to 159 per sample) in late February-early March, it should be noted that at this time the samples were pooled with those of the chicks and so it is not possible to state which bird(s) these oocysts actually came from. The treatment regime used for Jill and her chicks is given in Appendix II, Table 5.

Five other adults were sampled at various times during this season (Appendix II, Tables 6 & 7). A single oocyst was recorded on June 16th from a pooled sample from two adults and one juvenile, but only "Keith" and "Ngatiwai" were recorded as having shed any oocysts for the remainder of 1998. "Keith" was a special case and will be dealt with in detail later.

"Ngatiwai" shed very large numbers of oocysts in late September-early October which reduced to zero after a course of toltrazuril treatment. However, the oocysts reappeared in very large numbers again within 6 days (and responded again to treatment). This bird was recorded as being very ill over this time with suspected aspergillosis and was treated with flucytosine (Ancobon, Roche) and fluconazole (Diflucan, Pfizer).

(iii) Oocyst shedding from chicks/juveniles in the 1998-1999 breeding season

Clutch No.1: In the 1998-1999 season the first clutch of chicks hatched earlier than in the previous season (early December rather than January). Oocysts were found in small to moderate numbers towards the end of December when the chicks were about 22-23 days of age, shortly before fledging (Fig 2.3) (Appendix III, Table 1). The oocysts were first observed one day after the chicks were banded. Again, shedding was eliminated rapidly by the toltrazuril treatment.

Oocyst numbers again increased to low to moderate numbers in mid-January, after fledging when the chicks were approximately 6 ½ weeks of age. Shedding was eliminated by toltrazuril treatment for 5 days from January 20th.

The fledglings were separated from one another at about 7 weeks of age. One of the four, ("Missy"), began shedding extremely large numbers of oocysts at the beginning of February (Appendix III, Table 2). In this bird, oocyst numbers fell to zero after toltrazuril treatment but returned to extremely large numbers (up to approximately 86, 000 oocysts per gram) within four days which responded again to toltrazuril. There was a further very large increase in oocyst numbers in this particular bird in late February, but oocyst numbers again returned to zero with treatment. A second bird ("Rangi") also excreted oocysts during February: one episode consisted of very small numbers, another of very large numbers. These also responded to treatment.

The juvenile bird, "Missy", had further periods of shedding small numbers of oocysts in early March and mid-March but was consistently negative for the remainder of 1999.

A third juvenile bird,"Roz", shed moderate to very large numbers of oocysts towards the end of April, moderate to very large numbers again in early May, and no further oocysts for the remainder of the year.

In all cases, oocyst shedding was eliminated, at least temporarily, by toltrazuril treatment.

The other two December-born juveniles ("Rangi" and "Watson") were moved to Tiritiri Matangi Island on February 18th and so were not sampled after that date.

Clutch No.2: A second clutch of hihi hatched on 17th and 18th February and consisted of two chicks. They had two episodes of oocyst shedding in early March when they were approximately 14-19 days of age and responded to toltrazuril (Appendix III, Table 2). The

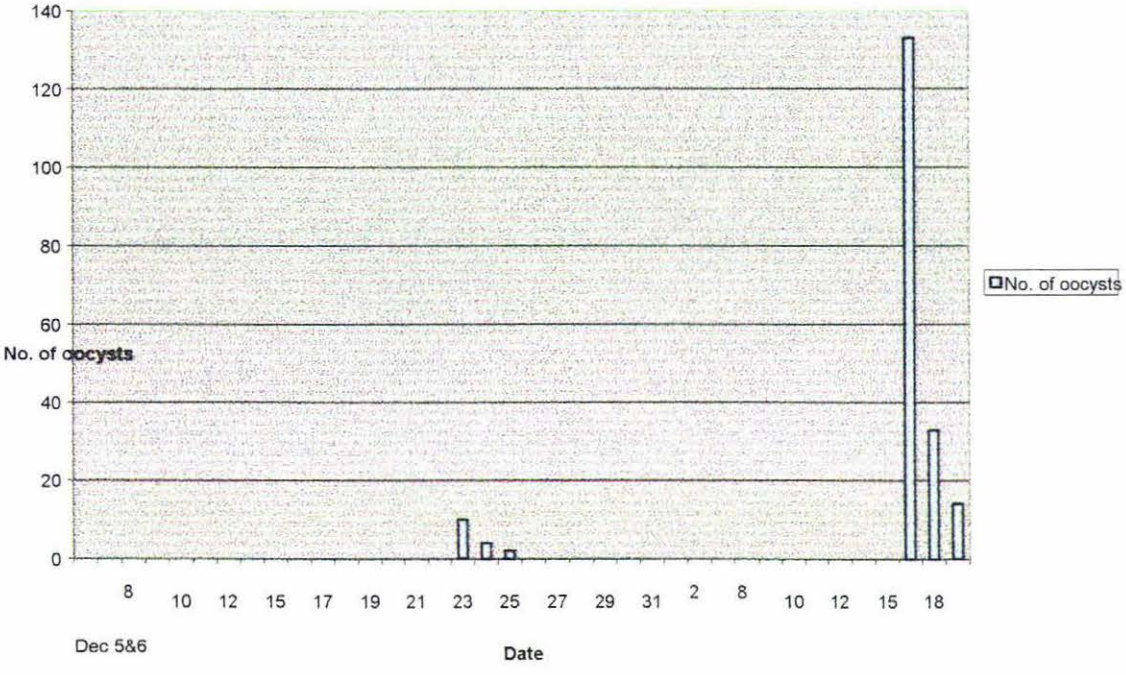
smaller of the two chicks was euthanased due to severe illness, on 10th March at 21 days of age (the cause of death was a severe systemic mycosis which was probably aspergillosis). The remaining chick, "Sally", shed large to very large numbers of oocysts in mid- to late March when it was approximately 33-35 days of age. This responded to toltrazuril. Shedding of very large numbers occurred in early April, large numbers in mid- to late-April, and large numbers again in September.

(iv) Oocyst shedding from adults in the 1998-1999 breeding season

Five adults were sampled from December 1998 through to December 1999. Three of these birds, "Ray", "Jill", and "Rachel", did not have any oocysts in any of the samples taken throughout those 13 months. "Ngatiwai" shed large numbers of oocysts mid-May but the counts quickly returned to zero (Appendix III, Table 3).

"Keith" had numerous episodes of shedding extremely large numbers of oocysts or "oocyst-like structures" (see later account).

Fig 2.3 Oocyst shedding by chicks during Dec 1998 and Jan 1999



(v) Shedding of *Capillaria* eggs

Most birds shed some *Capillaria* eggs at various times.

In both seasons, the chicks did not shed any eggs until approximately 5-6 weeks of age, and numbers ranged from 1-225 eggs per gram (epg) with most positive samples containing less than 60 epg (Appendix II, Tables 1, 2 & 3; Appendix III, Tables 1 & 2). The 1997-1998 clutch excreted *Capillaria* eggs far more often than the 1998-1999 December-hatched clutch. The former shed consistently before April and before separation from one another, but less often thereafter. Few eggs were passed by the 1998-1999 December-hatched clutch before separation from one another, and thereafter it was mostly one juvenile, "Missy", which passed *Capillaria* eggs intermittently throughout the year. The surviving bird from the 1999 February-hatched clutch, shed large numbers (up to 225 epg) frequently, particularly in May.

In 1998, the dam of the chicks shed *Capillaria* eggs in nearly all samples from mid-February and through March (Appendix II, Table 4). All the adults shed sporadically for the remainder of that year (Appendix II, Table 6). In the 1998-1999 season, there was sporadic shedding from December 1998 in all adults, particularly "Keith" and "Ngatiwai" early in the year (Appendix III, Table 3).

Dosing with ivermectin (Ivomec, MSD-Agvet) for 1-6 days appeared to eliminate the shedding of *Capillaria* eggs in some cases, but not all.

(vi) Oocyst shedding by the hand-reared bird, "Keith"

This hihi was found as an orphaned chick on Mokoia Island during the 1997-1998 breeding season and was subsequently hand-reared from the nestling stage. From then it was kept in captivity in a private aviary until it was moved to the N.W.C. at the end of July 1998 when it was approximately 8 months old. Throughout this rearing period it had

very little exposure to other birds. However, the bird was shedding oocysts before it was shifted to the N.W.C. and oocysts were present in the first sample collected 5 days after the translocation. After this, there were frequent episodes of oocyst shedding (Appendix IV, Tables 1 & 2). Initially, up to the end of October 1998, numbers ranged up to 227 oocysts per gram but after this time, numbers of oocysts (or oocyst-like structures), ranged up to approximately 78,500 per gram. Although these structures were very regular in shape, almost round, and morphologically resembled oocysts, the extremely large numbers present (which had not been encountered previously), along with the apparent good health of the bird, led to some doubt as to whether these were actually oocysts. Additionally, later samples contained these similar oocyst-like structures which appeared to be in various stages of collapse and distortion. Many of these appeared flattened and folded. Measurements taken showed these structures to be of a very similar size to oocysts shed by hihi at the N.W.C. (Appendix VII).

Shedding of the oocysts and the "oocyst-like structures" was eliminated by toltrazuril treatment, with numbers down to zero by the second day after treatment had finished. In order to study possible diurnal periodicity in oocyst shedding, faecal samples were collected at different times throughout the 24-hour day. There was no clear pattern in relation to time of day (Appendix IV, Table 1). Toltrazuril treatment was instituted and shedding was eliminated.

This bird received 2 courses of treatment with itraconazole (Sporonox, Janssen-Cilag), a human medication which was used for the prophylaxis and treatment of aspergillosis. The first course was given during July in anticipation of a shift to the N.W.C. (this was delayed) and the second course was given in late July-early August for the same reason (i.e. as a prophylaxis during and after translocation to the N.W.C.). On each occasion, oocyst shedding began during, or shortly after, the itraconazole course.

Extremely large numbers of oocysts continued to be shed until the end of 1998. In mid-January 1999, very large numbers were shed on one occasion, but otherwise the samples

taken were negative in 1999. The bird died on 18th February 1999 with chronic intestinal coccidiosis.

(vii) Pre-laying to post-hatching oocyst shedding by parents of chicks

Over the period from 2-4 weeks prior to egg-laying to 4-6 days after hatching during both breeding seasons, faecal examination of samples from the dam and sire of each clutch, revealed no oocysts (Appendix V).

2.3.2 Results of faecal examinations of hihi from other localities

Faecal material was collected intermittently from both Mokoia Island hihi and Tiritiri Matangi hihi. There were several positive samples from both locations at various times of the year but it was not possible to detect any pattern (Appendix VI).

2.3.3 Results of sporulation

(i) Sporulation methods

Successful sporulation was achieved by the following methods:
(i) Faeces placed in potassium dichromate and kept at 27°C.
(ii) Faeces placed in 2% sulphuric acid and kept at 27°C.
(iii) Faeces placed in 2% sulphuric acid and aerated at 27°C.

Most samples originating from the N.W.C. contained oocysts which did not show any signs of sporulation regardless of which methods were used (Fig 2.4). Small numbers of oocysts arising from pooled samples collected at the end of February-early March 1998 from "Jill" and her chicks, underwent partial sporulation in potassium dichromate at 27°C.

Some oocysts from Mokoia Island sporulated. These were from faeces collected in January 1998 and placed into potassium dichromate and kept at 27°C. Similarly, some oocysts from Tiritiri Matangi sporulated. Some of these oocysts were sporulated when the faeces were first examined. The cleaned and concentrated oocysts were placed in 2% sulphuric acid and kept at 27°C. Approximately two thirds of these oocysts sporulated.

An oocyst suspension from "Keith" (resident at the N.W.C.), which was aerated in 2% sulphuric acid at 27°C, had extremely low numbers of sporulated oocysts. These may have been present before processing.

A faecal mass from "Keith", which contained oocysts, was placed intact in the 37°C room for 2 days. When examined after this time, the oocysts were all collapsed, distorted and barely recognizable (Fig 2.5). None of these sporulated. A sample from "Ngatiwai" contained oocysts which were almost fully sporulated when they arrived for examination.

(ii) Sporulation Times

Times for sporulation could not be determined accurately, mostly because of limited success in achieving sporulation and lack of information on when samples were passed. However, a sample taken directly from a fledgling on Mokoia Island and sporulated in potassium dichromate at 27°C, was collected on 23/1/98 and processed on

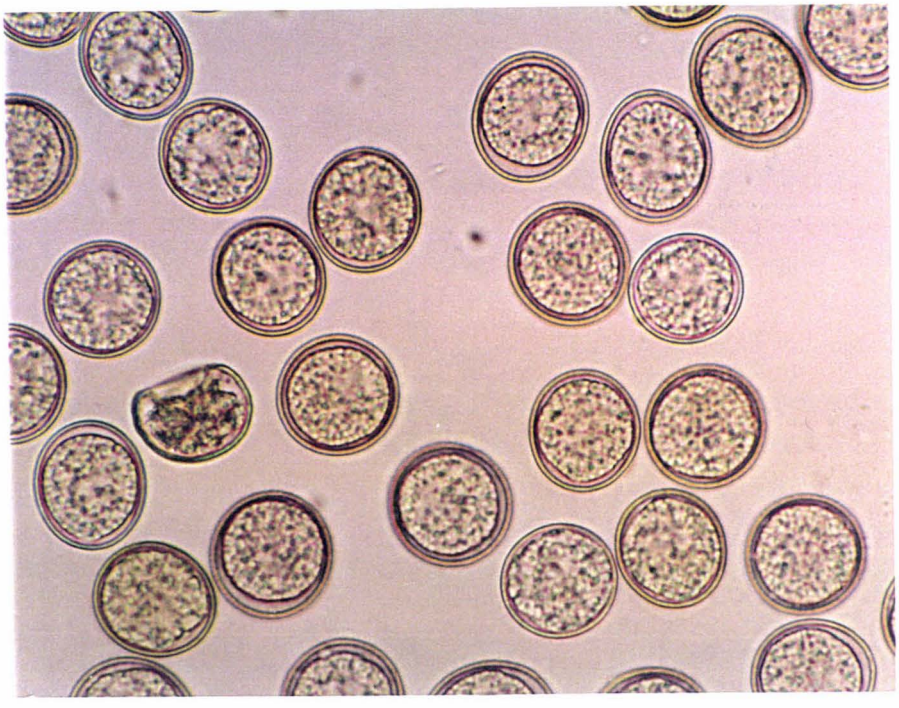


Fig 2.4 Photomicrograph of large numbers of unsporulated oocysts x850

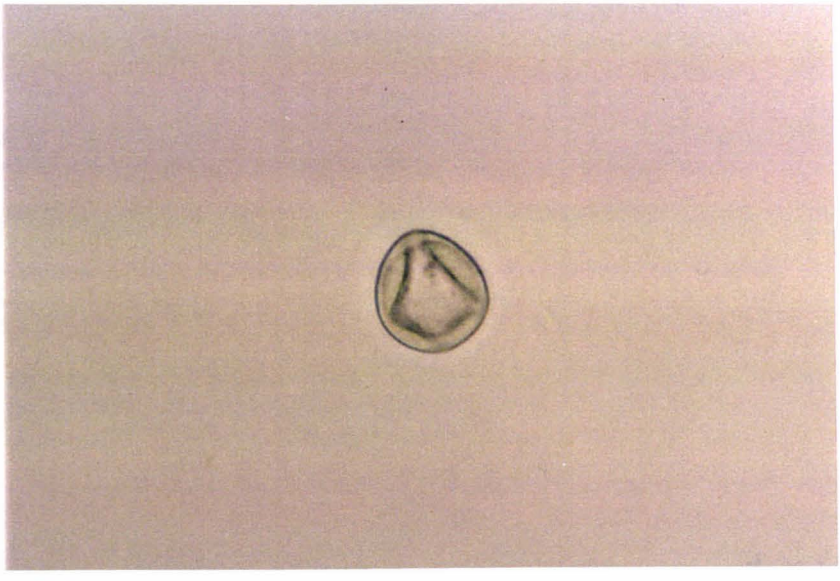


Fig 2.5 Photomicrograph of an early distorted oocyst which appears to be folding in upon itself x850

on 26/1/98. Some sporulated oocysts were found on 28/1/98 i.e. a sporulation time of five days. Samples received from Tiritiri Matangi Island which were received and processed on 3/2/99, contained oocysts, two thirds of which were sporulated three days after this date. The date of actual collection was not recorded. Other samples from these islands which contained oocysts which sporulated, did not add any additional information on sporulation times because it was not known, in most cases, when the faeces had been passed.
A composite sample from "Keith" which was collected on 30/10/98-2/11/98, 4/11/98-6/11/98, contained occasional sporulated oocysts (from very large numbers of oocysts) on 18/11/98. The earliest that these oocysts could have been passed was on 29/10/98. These oocysts therefore took 20 days or less to sporulate. The sample from "Ngatiwai" which contained oocysts, many of which were almost fully sporulated when the sample arrived, was collected on the morning of 11/5/99 and processed late morning on the 12/5/99. The earliest it could have been passed was 5.30-6pm on 10/5/99. The oocysts therefore took less than 41 hours to get to this stage of sporulation.

2.3.4 Oocyst morphology

(Detailed data on oocyst morphology are given in Appendix VII).

The oocysts were placed into two main groups based on the general appearance of the oocyst shape in conjunction with an arbitrary measurement of length-width difference. Subspherically shaped oocysts comprised one group and ellipsoid oocysts comprised the other. While this difference in shape was usually quite obvious, for consistency it was decided that those oocysts < 3 µm length-width difference be assigned to the subspherical group and those with > 3 µm length-width difference be assigned to the ellipsoid group. Therefore, the two main types were: Type A- those of a subspherical shape; Type B- those of an ellipsoid shape.

Analysis of variance (Statistix 7, Analytical Software, Tallahassee, Florida) showed that these two types differed significantly in length ($p<0.001$) and length-width ratios ($p<0.001$) but not widths ($p>0.05$).

Table 2.1 gives the descriptive statistics on Types A & B.

Table 2.1: Descriptive statistics of the two types of oocysts

	Type A length	Type A width	Type A length/width ratio	Type B length	Type B width	Type B length/width ratio
N	26	26	27	21	21	21
Lo 95% CI	19.338	17.961	1.0523	23.515	18.924	1.2092
Mean	20.031	18.714	1.0728	24.461	19.627	1.2484
Up 95% CI	20.724	19.467	1.0933	25.407	20.330	1.2877
SD	1.7155	1.8637	0.0508	2.0789	1.5443	0.0862
SE Mean	0.3364	0.3655	9.959E-03	0.4537	0.3370	0.0188
Minimum	16.400	15.360	1.000	18.800	15.440	1.1136
Maximum	22.960	22.080	1.1622	28.400	21.840	1.4381

Within Type B there was one possible outlier, as demonstrated by a Box and Whisker Plot, and this was the oocyst measuring 15.44x18.8 µm. In addition, there were two oocysts which were not classified within the two main types because their dimensions were markedly larger: 28.4µmx26.72µm, 27.6µmx25.04µm. These both originated from Mokoia Island.

Of the Type A oocysts measured, 10 originated from Tiritiri Matangi Island, 13 from the N.W.C. , and three from Mokoia Island. Of the Type B oocysts measured, 16 originated form Tiritiri Matangi Island, three from Mokoia Island, and two from the N.W.C.

Preliminary morphological characteristics

All measurements are given in micrometres (µm) and presented as mean ±SD followed by the range in parentheses.

Type A (Fig 2.6)

Description

Sporulated oocyst subspherical, 20 ± 1.7 x 18.7 ±1.9 (16.4-23 x 15.4-22.1), shape index (length/width) 1.1 ±0.05 (1-1.2) with an indistinct micropyle and no micropylar cap. Sporocysts ellipsoid, 14.9 ±1.9 x 9.2 ±1 (11.3-18.1 x 7.1-11.6). Sporocyst residuum present. Sporozoites lie lengthwise in sporocyst.

Type-host: *Notiomystis cincta*, hihi (stitchbird).

Locality: National Wildlife Centre, Mount Bruce; Mokoia and Tiritiri Matangi Islands.

Prevalence: Unknown.

Site of infection: Unknown; oocysts found in faeces.

Sporulation time: Unknown.

Type B (Fig 2.7)

Description

Sporulated oocyst ellipsoidal, 24.5 ± 2.1 x 19.6 ± 1.5 (18.8-28.4 x 15.4-21.8), shape index (length/width) 1.2 ± 0.08 (1.1-1.4) with an indistinct micropyle and no micropylar cap. Sporocysts ellipsoid 16.8 ±2.8 x 10.6 ± 1.3 (11.4-21.4x 8.4-14.3). Sporocyst residuum present. Sporozoites lie lengthwise in sporocyst.

Type-host: *Notiomystis cincta,* hihi (stitchbird).

Locality: National Wildlife Centre, Mount Bruce; Mokoia and Tiritiri Matangi Islands.

Prevalence: Unknown.

Site of infection: Unknown; oocysts found in faeces.

Sporulation time: Unknown.

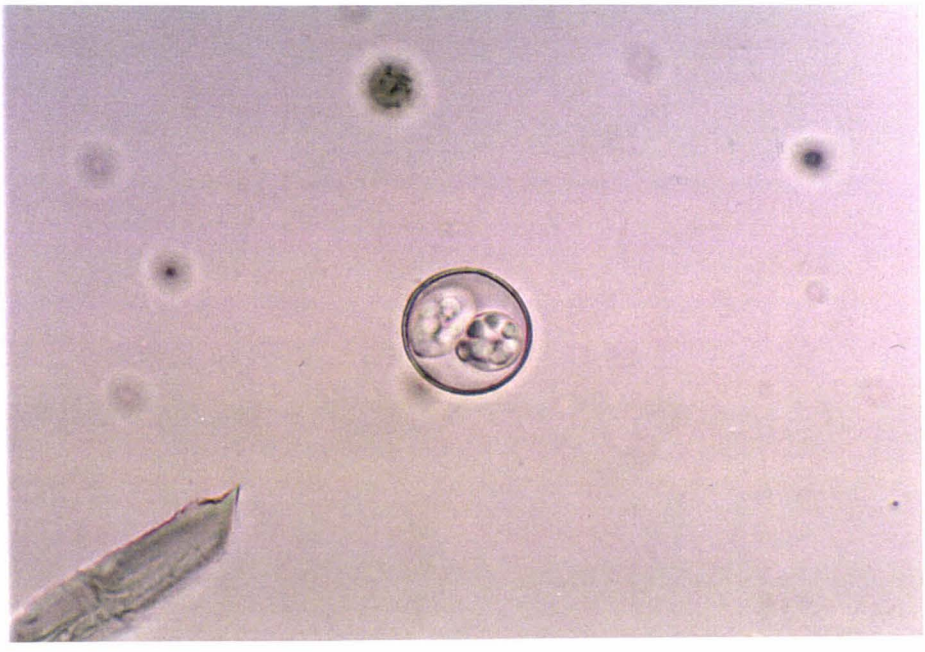


Fig 2.6 Photomicrograph of a sporulated, subspherical Type A oocyst x850

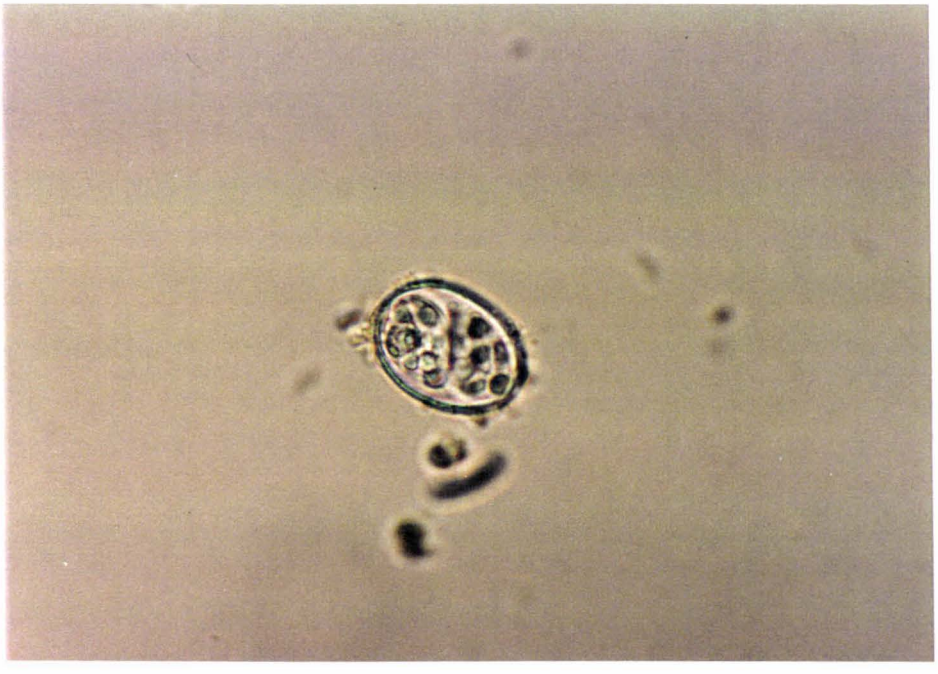


Fig 2.7 Photomicrograph of a sporulated, ellipsoidal Type B oocyst x850

2.4 DISCUSSION

This study provides the first extensive set of data showing oocyst shedding by hihi over a period of time, particularly shedding by birds in captivity. This has enabled some preliminary observations to be made on the relationship between shedding, the life history of the birds and the effect of external factors. The study has also provided information on oocyst morphology which is necessary to investigate further the species of coccidia involved.

The oocyst counts revealed some patterns in oocyst shedding, and some of these recurred given similar conditions. However, there were many variables relating to collection of samples, storage, processing and recording which could have affected the oocyst counts recorded and complicated their interpretation.

The method of collection of samples varied and this was an ongoing problem. Samples from the N.W.C. were mostly collected from roost boxes, rather than directly from birds. Other collection sites were sometimes used e.g. collection from transport boxes. When the samples were gathered indirectly from the bird, especially from an outdoor site, there was the possibility of faecal contamination from other birds such as those which had previously occupied that aviary or birds passing faeces through the aviary mesh e.g. sparrows. The time of the day at which the sample was passed might also influence the count as diurnal periodicity of oocyst shedding in coccidia-infected passerines, has been recorded by Box (1975) and Grulet *et al.* (1986). Another complication was the periodic administration of toltrazuril which was not under the author's control.

The laboratory techniques used needed to be simple, repeatable, inexpensive, and give meaningful, comparable results. The standard methods used in the veterinary parasitology laboratory at Massey University were employed because the techniques were known to work, they were familiar to the staff and the equipment needed was present. Any changes required were implemented as the research progressed. Box (1970, 1975, 1977)

sporulated isosporan-type oocysts originating from canaries, in 2.5% potassium dichromate at room temperature. Lindsay and Blagburn (1994) reported that in mammalian *Isospora* species, temperatures >40°C or <20°C inhibit sporogony and rapid sporulation of oocysts (<16h) occurs at temperatures between 30°C and 37°C.

There were many stages during the processing for counting and during the counting itself, where inaccuracies affecting the number of oocysts reported could have arisen e.g. loss of oocysts during centrifugation and separation of the supernatant. Operator inexperience might have been a factor as the author was not experienced in handling oocysts from any species at the beginning of this work.

In order to expedite the routine counting process, only the contents of half the microscope slide were counted (using a scored microscope slide) and this figure doubled to give the result. This assumed that the oocysts were evenly distributed over the slide, whereas in fact this was not necessarily so. In many cases it was seen that the oocysts were clumped on the slide. However, the consistency in method enables valid comparisons to be made.

Another area of inconsistency was in the recording methods used. When the author was absent, subjective methods were used to record the faecal flotation results e.g. a scale of +, ++, +++, ++++ was sometimes used instead of a real number count. Results were initially recorded as number of oocysts per sample but this changed in August 1998 when weighing of samples and recording as number of oocysts per gram, was initiated (in an attempt to make the results more comparable as the quantity of sample submitted could vary substantially).

The results have shown that in both seasons all chicks had at least two periods of oocyst shedding: one before fledging while in the nestling stage and one or more post-fledging. The earliest recorded shedding was at 9 days of age. Post-fledging, a further period of high oocyst shedding was between 6.5- 8 weeks of age. This timing therefore corresponds to the months of December through to February, depending on when the clutch hatched.

Some chicks had further periods of shedding at various times throughout the remainder of the year.

Of the adults, only two birds, "Keith" and "Ngatiwai", recorded any shedding of substantial numbers of oocysts. The female "Jill", was recorded as having shed oocysts but this was at times when the bird was housed with her chicks and individual samples were not identified. This meant that the oocysts might have come from "Jill" or any of her chicks.

"Ngatiwai" shed large numbers of oocysts during September and October 1998. "Keith" shed large numbers during late October, November and December of 1998. This pattern is consistent with the idea that oocyst shedding increases during, and prior to, the breeding season. Hihi can suffer severe "stress" at this time as they have a particularly aggressive and promiscuous sex life (Castro, 1995). However, high numbers of oocysts were not recorded at this time from samples of the other adult male or by "Ngatiwai" during the following (1999) breeding season, although sampling was not frequent. No conclusions can therefore be drawn on the effects of the breeding season on oocyst shedding by the adult males. Another possible explanation for "Ngatiwai's" shedding over September and October 1998 is the ill health that the bird was experiencing at the time. This ill health might have been the cause of the shedding (due to concurrent disease such as aspergillosis) or might have been a result of the coccidial infection, although this latter possibility is assumed to be less likely because the other adult birds generally did not have similar episodes.

The numbers of oocysts shed varied greatly with numbers up to 86,000 per gram having been recorded for the chicks and up to 78,500 oocysts per gram for "Keith", the hand-reared bird. "Ngatiwai" shed up to 3,370 oocysts per gram. In general, "Keith" had many more samples recorded with numbers in the order of several thousand oocysts per gram than any of the chicks or other adults, which suggests this bird did not develop immunity

to the coccidia as other birds did. The comparable chick samples were from the post-fledging period; oocyst shedding by the nestlings was generally of much lower numbers.

In all cases, oocyst shedding was eliminated, at least temporarily, by treatment with toltrazuril. Oocyst numbers were generally down to zero by the 5th day of treatment, except with "Keith" where this sometimes took up to 7 days. However, oocyst numbers sometimes rose again quickly after treatment ended. On occasion, numbers were up one day after a zero recording. The oocyst-like structures shed by "Keith", about which there was some uncertainty as to whether they were oocysts (because of the large numbers present and the difficulties experienced in inducing them to sporulate), were eliminated by toltrazuril, which strongly suggests that the structures were indeed oocysts.

Shedding of oocysts occurred after treatment with itraconazole (used as a treatment and as a prophylaxis for aspergillosis) in "Keith" (the only bird to have this drug during the study period). It is possible that the shedding was somehow initiated by the drug, although there is no mention of an immunosuppressive effect in the literature on itraconazole. Alternatively, the shedding might have been unrelated; it was administered on only two occasions during the research.

An attempt was made to investigate the possible occurrence of diurnal periodicity in oocyst shedding as reported by Box (1975). Unfortunately, no conclusions can be drawn from the results because samples were taken at different times of the day on too few occasions, and, toltrazuril treatment was started during the periods of sample collection.

Because of irregular and infrequent sampling schedules it is difficult to draw any firm conclusions regarding oocyst shedding from free-living hihi on the islands. However, positive samples were recorded from Mokoia in January and February 1998 and from Tiritiri Matangi in January and February 1999 with very low numbers from the latter in August 1999. Numbers of over 1000 oocysts per sample were recorded from Mokoia and much lower numbers from Tiritiri Matangi.

There did not appear to be any relationship between *Capillaria* egg shedding and oocyst shedding throughout the study period. Chicks did not shed any *Capillaria* until 5-6 weeks of age. Ivermectin treatment eliminated nematode egg shedding.

The difficulty in sporulating hihi oocysts was a major problem in this study. There are many factors which could have interfered with successful sporulation in the laboratory. Many birds had been given various drug treatments, which could have affected oocyst viability. Distorted and collapsed oocyst-like structures were found occasionally several days after itraconazole treatment but this was not consistent. On several occasions oocysts were found similarly distorted after periods of time in the sporulation media or, in one case, after having been left as a faecal mass at 37 °C for two days. It is known (Joyner and Davies, 1960) that the solutions commonly used for flotation (particularly sodium chloride) can have a deleterious effect on oocysts due to osmotic effects causing dehydration. Saturated sodium chloride solution was used for flotation in this research but the time during which the oocysts were exposed to this was kept as short as possible (a maximum of 30 minutes). On several occasions, when it was thought to be highly likely that the sample contained oocysts, this step was left out. However, this did not influence sporulation.

Many aspects of the handling of the faeces before processing may have had an effect on sporulation. Oocyst survival is greatly affected by temperature and moisture (Fayer, 1980). Dessication can reduce oocyst viability, as can high temperatures (Yilmaz and Hopkins, 1972), although the latter is less well-documented and appears less consistent. Dessication might very well have been an issue on occasion as some of the hihi samples looked dry when they arrived at Massey University, especially those which had come from islands and so could have been passed and collected many days previously. Low environmental temperatures might not be detrimental, although it has been suggested (J. Barta, pers. comm.) that refrigeration of faecal samples can inhibit subsequent sporulation. These are aspects which might not have always been considered or had appropriate

allowance made for, during transport and storage of the samples, especially if there was a delay in either receiving the samples (e.g. in dispatch or transport) or in processing (e.g. when other work commitments took priority). Because of the possibility of refrigeration being detrimental, samples were no longer refrigerated at the N.W.C. or at Massey University before processing, after August 1998.

Oxygen is necessary for sporulation of oocysts to occur. Therefore, if the sporulation medium is too deep relative to the surface area, oxygen could be a limiting factor. Particular care was taken to address these issues during the study and it is unlikely that lack of oxygen was involved in the sporulation difficulties.

In the light of the fact that the oocysts from other locations sporulated relatively readily and the fact that the techniques used have been successful with coccidia from other species, it seems likely that the problems of unsuccessful sporulation were associated with the managed environment at the N.W.C. rather than with the techniques used in the laboratory. Alternatively, it is possible that the coccidia species involved has some particular or unusual requirement for sporulation. Subsequent to this research, further oocysts from the N.W.C. were sporulated in 2% potassium dichromate at room temperature (B. Adlington, pers. comm.). Sporulation time was prolonged (approximately 21 days) but there was no variation from the standard techniques used by the author. The reasons for the variability in results are not apparent.

In order to encourage sporulation *in vitro*, factors such as the temperature and sporulation media were varied. Given the generally cool and damp environment at the N.W.C., the conditions provided in the laboratory seemed appropriate.

Further work is required to identify the source of infection of captive birds. It is possible that the adults (specifically the parents of the chicks) are harbouring sub-clinical infections and excreting and contaminating the environment leading to infection of the chicks. This contamination could be occurring over the breeding season. However, the limited data

presented here relating to oocyst shedding by the parents in the month immediately prior to, and during the time of laying and hatching, does not support this possibility (i.e. there is no shedding). Alternatively, the parents may have contaminated the environment (outside of the nest as adults rarely defaecate in nests) after hatching of the chicks, immediately prior to chick infection. This study did not gather sufficient samples, specifically from the parents, to determine their contribution to infection. Further studies should therefore follow oocyst shedding from the parents, more closely, prior to and after hatching, in order to give more conclusive results. The situation may be similar to that which is seen in neonatal porcine coccidiosis where the sow had long been considered a logical source of infection but experimental studies failed to confirm this (Lindsay and Blagburn, 1994). *Isospora suis* was not found in the faeces of sows from farms with a history of porcine coccidiosis although in one study faecal examinations were performed immediately prior to farrowing, at farrowing, and post-farrowing (Stuart and Lindsay, 1986). Piglets nursing these sows developed coccidiosis and passed *I. suis* oocysts in their faeces. From this and other work, it was concluded that once *I. suis* is established on a farm, it is probably maintained by piglets which acquire their infections from *I. suis*-contaminated farrowing crates (Lindsay and Blagburn, 1994). In the hihi therefore, it is possible that the infection is maintained in chicks and juveniles and the oocysts remain viable in the environment for a long period of time. Further research should therefore include studies on hihi oocyst viability.

CHAPTER 3

TRANSMISSION EXPERIMENTS

3.1 INTRODUCTION

For many years it has been generally believed that coccidia are highly host-specific. Levine (1974) has been a proponent of this view and Svobodová (1994) concurred by recommending that monoxenous coccidia be considered genus-specific unless there are positive tests of infectivity for other genera. Exceptions are known to occur e.g. *Eimeria dispersa* from turkeys (*Meleagris gallopavo*) can develop in the pheasant (*Phasianus colchicus*) and other members of the order Galliformes (Doran 1978). Box (1970) attempted to transmit sparrow *Isospora* oocysts to a canary (*Serinus canarius*) but no infection resulted. Khan and Desser (1971) also attempted to transmit *Isospora* oocysts from the faeces of evening grosbeaks (*Hesperiphona vespertina*) to canaries but again without success.

In order to accurately determine a new species, the complete life cycle of a parasite needs to be known and cross-transmission studies performed (Ball *et al*, 1998). Insufficient work has been carried out on most species of *Atoxoplasma* to elucidate their exact life cycle and their species-specificity, nevertheless, the generalizations concerning the species-specificity of coccidia are considered to hold. Because of the serious impact that the coccidial infection in hihi has had at the National Wildlife Centre (N.W.C.), it was considered necessary to investigate the epidemiology of infection as far as possible. Little has been determined previously regarding the source of infection and the possibility exists that species other than hihi are involved in carrying and transferring the organisms. The present investigation was divided into three separate transmission experiments, with different objectives as follows:

1. The starling (*Sturnus vulgaris*) experiment. Starlings have been found to be infected with coccidia (Batyrshina and Kazykhanova, 1976). In a study by Mazgajski and Kedra (1998), *Isospora* species were found in 67% of starling broods in Poland. Coccidia have also been observed in starling intestinal epithelium in New Zealand (Alley, pers. comm.). Starlings were the species chosen for the present experiment because of their abundance around the N.W.C. and the likelihood that their faeces would fall through the aviary mesh into the enclosures. However, in a study performed by Wagner and Ruedi (1981) on the endoparasite status of the aviary birds in the Basle Zoo, Switzerland, it appeared that the transmission of nematodes, but not coccidia, was occurring largely via wild birds. In the present study, faeces were to be gathered from wild starlings and these samples examined in order to ascertain whether coccidial oocysts were present. Artificially-raised parasite-free starling nestlings were to be divided into two groups: a control and a treatment group, with the treatment group inoculated with the sporulated form (oocysts to have been sporulated in the laboratory) of the wild-harvested starling oocysts and the control group inoculated with water.

The objectives were:

(i) To determine whether or not oocysts were being excreted by starlings, and, if so, to describe and document them, and compare them with those of the hihi.

(ii) To determine whether a patent infection can be induced artificially in starlings using wild-harvested starling oocysts, and if so, to see whether this infection is pathogenic to starlings.

(iii) If successful, this work could then serve as a model for transmission studies using hihi oocysts.

If starling oocysts could not be harvested or sporulated, sporulated hihi oocysts were to be used for inoculation into the starlings. The procedure was to be the same as with the starling oocysts. The objectives were:

(i) To determine whether a patent infection can be induced artificially in starlings using hihi oocysts, and if so, to see whether the organisms are pathogenic to starlings.

(ii) If successful, this work could then serve as a model for further transmission studies.

2. The hihi experiment. A single female hihi became available for experimental purposes and so a transmission experiment using hihi oocysts, was undertaken. Hihi oocysts, which had originated from the N.W.C. and sporulated in the laboratory, were inoculated into this bird. The bird was ultimately to be euthanased, either at the first clinical indication of disease or suffering, or, if this did not occur, euthanasia was to occur after adequate time had passed to enable infection with the oocysts to establish, if this was going to happen. This assessment was to be based on the results of the faecal and blood examinations and could not be pre-determined. The objectives were:

(i) To determine whether a patent infection can be induced artificially in a hihi using hihi oocysts sporulated in the laboratory, and if so, to see whether this infection is pathogenic to the hihi.

(ii) If infection was successful, to determine the pre-patent period.

(iii) If infection was successful, to determine whether or not there are coccidial forms evident in the blood and, if so, the time taken for these stages to become apparent.

3. The finch experiment. Systemic protozoal disease has been documented in zebra finches (*Poephila guttata castanotis*) (Helman *et al*, 1984). In the present experiment, juvenile zebra finches were to be inoculated with sporulated hihi oocysts after coccidiocidal treatment and testing to ensure they were coccidia-free. The birds would be euthanased in pairs (one treatment and one control bird) at regular intervals and necropsies performed. The objectives were:

(i) To determine whether a patent infection can be induced artificially in zebra finches using hihi oocysts, and if so, to see whether this infection is pathogenic to finches.

(iv) If successful, this work could then serve as a model for transmission studies using other bird species as recipients.

Finches were chosen because they are easily obtainable and easily kept in captivity.

3.2 MATERIALS AND METHODS

3.2.1 Starling Experiment

(i) Examination of wild starling faeces

During the 1998 nesting season, a mist net was erected over one of two large entrance-ways into a machinery and implement shed at the N.W.C. which was frequented by wild starlings. Faecal samples were collected from captured birds and taken to Massey University where they were examined for the presence of oocysts using the procedure described previously (Chapter 2.2).

(ii) Acquisition of eggs

Twelve nest boxes, based on a design proposed by Coleman (1974), were constructed and placed at three sites: in a large backyard of a city residential property, in the garden of a property in the country, and on a North Island hill country farm. Placement was during the very early stages of the nesting season (early August) to allow time for the birds to accept the structures in their vicinity. The boxes were observed for signs of nest-building activity and once this occurred they were monitored daily for egg-laying. As close as possible to 12 days after the laying of the last egg in the clutch, the eggs were all removed.

(iii) Incubation of eggs

The eggs were candled and those containing live embryos or those of an uncertain viability, were transferred to a sterile incubator in a quarantine section of the veterinary tower at Massey University. The incubator was maintained at a temperature of approximately 37.1-37.2 °C and a humidity of approximately 60-65 and the eggs were automatically turned. At approximately 24-48 hours prior to hatching, the settings were changed to 36.5 °C and a humidity of 70 and the egg turning was stopped.

(iv) Raising of parasite-free nestlings

Hatchlings produced were transferred to a separate quarantined room in the veterinary tower where they were kept in batches of 4-6 birds in cardboard boxes, mimicking clutches. Heat lamps served as the heat source and an automatic diurnal light-dark system was in operation. The birds were fed on lean minced ox heart, a commercial cereal mix (Pronutra, National Brands Ltd, Wadeville, South Africa), a commercial honeyeater mix (Wombaroo, Wombaroo Food Products, Adelaide, Australia), and mealworms, with water provided via a syringe. Feeding was by individual placement of the feed in the mouths via forceps until begging stopped. The nestlings were fed every 1-1.5 hours between 6.30am and 9.30pm during the first week, increasing to two hourly intervals at one week and at gradually increasing time intervals thereafter. Good standards of hygiene were maintained and all feeding utensils were kept clean using soapy water and Milton's solution (sodium dichloroisocyanurate).

In addition, two batches of artificially incubated starling hatchlings were provided by the staff at the NWC.

3.2.2 Hihi Experiment

(i) Source of experimental bird

A nestling female hihi ("Laurie") was recovered from Mokoia Island after it was found in the nest with a damaged leg. The bird was brought to Massey University where attempts to mend the leg failed and so amputation was performed. This bird was thought to be non-viable in the wild, and it was not considered to be suitable to hold in captivity for either advocacy or breeding. The bird was therefore made available for experimental purposes.

(ii) Care of experimental bird

The bird was housed in a quarantined room in the veterinary tower which was cleaned with a sodium hypochlorite solution and modified to provide a suitable and safe habitat. The bird was fed once per day, in the morning. The diet consisted of fruit (e.g. bananas, grapes, oranges, pears and feijoas), Wombaroo honeyeater mix and water. The feed dishes were cleaned and sterilised in Milton's solution after use. Diurnal day-night lighting patterns (14 hrs light and 10 hrs dark) were followed throughout the experiment.
The bird's general demeanour, perching ability, movement, grooming, state of alertness, brightness and clearness of eyes, and cleanliness/soiling of cloaca were observed and recorded three times daily.

(iii) Sampling

Faecal material was collected in two large plastic trays which were placed beneath the bird's most commonly used roosting sites. These trays were removed and replaced one-three times per day and the faecal material gathered was examined for parasites, particularly oocysts, via a faecal float. The time of day of each faecal collection was noted in order to detect any pattern in oocyst excretion, if oocysts were found. Faecal sampling was undertaken as shown in Table 3.2.

On day three, the bird was blood sampled via a brachial vein, and blood smears made and stained with Diff-Quik prior to microscopic examination.

(iv) Inoculation

On day four, at 11.45am, the bird was inoculated orally via a crop tube, with 0.8ml of a suspension containing 1667 sporulated oocysts per ml (approximately 1334 sporulated oocysts).

3.2.3 Finch Experiment

(i) Experimental birds

Eleven juvenile zebra finches (< 6 months of age) were obtained from a large aviary in Palmerston North. Three further finches were bought from a pet shop, giving a total of 14 birds. All of these birds appeared clinically healthy.

For 10 days before treatment, the birds were housed in a large mesh-fronted cage measuring approximately 110 x 80 x 80 cm. This cage was placed within a bird-proof enclosure within a shed. There was no opportunity for contact with wild birds or the faeces of wild birds. Feeders containing bird seed, shell and water, were made available in the cage with cleaning and refilling of dishes occurring twice per day.

(ii) Pre-inoculation sampling and treatment

Pooled faecal samples were collected every morning from plastic sheeting which was placed on the floor of the finch cage. Faecal floats were performed on these samples and the results recorded. In order to eliminate any pre-existing coccidial infections, all of the birds were treated with an anticoccidial drug, toltrazuril (Baycox solution 2.5%, Bayer), at a dose rate of 15mg/kg, for three consecutive days following four days of sampling.

(iii) Preparation of inoculum

The inoculum was prepared from sporulated oocysts obtained from hihi on Tiritiri Matangi Island. The faeces were collected from nest boxes on the island on 21/2/99, received at Massey University on 3/2/99, and processed on the same day.

The oocysts were sporulated in 2% sulphuric acid at 27 °C and washed free of the sporulation medium by dilution and centrifugation in 2-4 changes of distilled water. The concentrated suspension of oocysts in distilled water was stored at 4 °C until required. The number of oocysts present were estimated using a haemocytometer.

(iv) Inoculation

Immediately before inoculation, the birds were arbitrarily divided into two groups which were housed separately: a treatment group of 8 birds and a control group of 6 birds. Each bird was individually identified using its leg band or plumage, and weighed. Each finch in the treatment group was inoculated with 0.1-0.2ml oocyst suspension which contained a total of 47-95 sporulated oocysts, and the control birds received 0.1-0.2ml of water. Pooled faeces from both the control and treatment groups were collected every morning, the faeces weighed, faecal floats performed and the results recorded.

(v) Euthanasia and necropsy

Table 3.1 shows the number of birds from each group that were euthanased post-inoculation.

Table 3.1: Time Intervals of Euthanasia

	Day Post Inoculation				Total
Group	0	5	10	15	20	
Treated	0	2	2	2	2	8
Control	2	1	1	1	1	6

Euthanasia was carried out using an overdose of inhaled halothane and a necropsy was performed (see Appendix VIII).

After a minimum of 48 hours fixation, all tissues were trimmed and placed into cassettes for histological processing. The entire gastrointestinal tract was processed for histological examination and the segments were orientated so that proximal and distal regions could be identified. The tissues were routinely processed, embedded in paraffin and sections were cut at 3 μm. The slides were stained with haematoxylin and eosin before examination under a light microscope.

3.3 RESULTS

3.3.1 Starling experiment

No oocysts were found in faeces recovered from wild starlings and so inoculation with starling oocysts could not be undertaken.

Inoculation of parasite-free starling chicks with sporulated hihi oocysts, could likewise not be undertaken because of the unavailability of sporulated hihi oocysts when the parasite-free chicks were ready for inoculation.

Of the 18 starling chicks which hatched, three died during raising. The remaining 15 chicks were euthanased by an overdose of inhalation halothane approximately 20 days after the first hatch, when it was realised that inoculation could not occur. Necropsies were performed on all birds and there were no significant findings.

3.3.2 Hihi experiment

(i) Daily monitoring

The results of daily monitoring of "Laurie" are shown in Table 3.2. The bird died on day 6 of the experiment.

Table 3.2: Results of daily monitoring of "Laurie"

All results are shown as number of oocysts per sample/ number of *Capillaria* eggs per sample

Day	Action	Faecal	Results		Blood Results	Observations
		am	early pm	mid-pm		
1				0/0		B/A, eating, very friendly, very mobile, watery droppings
2			0/0			B/A, eating, mobile
3	Blood tested 2pm	0/0	0/0	0/1	No blood parasites	B/A, eating, mobile
4	Inoculated with oocysts 11.45am		20SP/0	17USP/1		B/A, eating, mobile
5		78USP[a]/1	0/0	6USP/0		pm- a little subdued, eyes partially closed some of the time, a little fluffed up, passed very little faecal material all pm
6						Found dead early am. Consumed a moderate amount of food during the night

B/A= bright and alert

SP= sporulated oocysts

USP= unsporulated oocysts

[a] = many oocysts looked unhealthy with imperfect walls and some indentations

(ii) Necropsy Results

The necropsy showed the cause of death to be systemic aspergillosis (for the full necropsy report refer to Appendix IX), with *Aspergillus fumigatus* infection of the lungs, liver, bursa, myocardium and peritoneum. Many of these were long-standing, fibrosing lesions indicating they had been present for a number of weeks. There were also recent mycotic lesions, indicating a recrudescence of infection. There was no evidence of coccidial infection grossly or histologically. A faecal float of the intestinal contents revealed 124 unsporulated oocysts and 1 *Capillaria* egg.

3.3.3 Finch experiment

(i) Daily monitoring

Some *Isospora*-type oocysts were present in faeces sampled from the finches before toltrazuril treatment. Many of these oocysts were already sporulated. After a three day course of toltrazuril, there were no oocysts shed for two days, after which time the birds were inoculated with oocysts. All faecal samples thereafter were negative for oocysts, up to and including day 20 when the last birds were euthanased (Appendix IX, Table 1).

(ii) Necropsy Results

There was no evidence of coccidial infection in any of the finches at necropsy. There were, however, 8 cases with hepatic foci of inflammatory cell infiltration (both mononuclear cells and mixed inflammatory cells in different cases), ranging from mild to severe, and several of these were multi-focal. Both control and inoculated birds were affected with these lesions. Other findings included multi-focal areas of inflammation in the heart of one case and a focus of fungi in the gizzard of another.

(See Appendix IX, Table 2 for an example of the histopathology results).

3.4 DISCUSSION

3.4.1 Starling Experiment

Neither part of this experiment could be completed and so no conclusions can be drawn. Failure to complete the experiment was due to the lack of a suitable inoculum when the nestlings were ready to be inoculated. If further work in this area is contemplated, more extensive faecal sampling of starlings might need to be undertaken to find sufficient coccidial oocysts for inoculation. Mazgajski and Kedra (1998) found *Isospora* species in 67% of starling broods and Batyrshina and Kazykhanova (1976) found 33.3 to 45.1% of the starlings they examined to be infected with *Isospora.* Although environmental conditions in New Zealand are different, it could be expected that similar proportions of starlings in New Zealand would be infected. We examined the faeces from only a small number of birds, and birds only from one area.

3.4.2 Hihi Experiment

The presence of oocysts (both sporulated and unsporulated) in the faeces of "Laurie" on the day of inoculation and the following day, were most probably a result of the passage of the oocysts directly through the gastrointestinal tract unchanged, rather than products of an infection. The fact that many of the oocysts passed the following day were unhealthy-looking, adds credence to this assumption because freshly formed and passed oocysts should have a healthy morphology. Box (1977) found that the pre-patent period for *Atoxoplasma serini* in canaries was 9-10 days, whereas the pre-patent period of the coccidial organism(s) in the hihi is unknown. It therefore seems very unlikely that one day (or less) after inoculation would be sufficient time for oocysts to be passed in the faeces. It is likely that the recrudescence of the aspergillosis in "Laurie" was triggered by the stress involved in the shift to the research facility, all the associated changes in living conditions, and the increased handling.

3.4.3 Finch Experiment

There was no evidence of transmission of infection via coccidial oocysts from the hihi to the finches. We can therefore conclude, with reasonable certainty, that this particular hihi coccidium is not infectious to zebra finches. It is notable that isosporan-type coccidial oocysts were present in the faeces of the finches prior to treatment, which is consistent with previous recordings of coccidia in zebra finches (Blanc & Grulet, 1985; Prattis *et al.*, 1990).

Further transmission experiments would need to be performed using avian species which are more closely related to the hihi e.g. the tui or bellbird, in order to investigate more fully the species-specificity of the organism. Hihi can not be used because of their vulnerable conservation status.

CHAPTER 4

PATHOLOGY

4.1 INTRODUCTION

The pathology of atoxoplasmosis, or infection with *Atoxoplasma*-like organisms, has been described by many authors in a variety of avian species (Helman *et al.*, 1984; Cooper *et al.*, 1989; Partington *et al.*, 1989; Panigahy and Senne, 1991; McNamee *et al.*, 1995; Norton *et al.*, 1995; Rossi *et al.*, 1997; Martinez and Muñoz, 1998; Ball *et al.*, 1998; Quiroga *et al.*, 2000). The most common gross findings were hepatomegaly, splenomegaly and thickening of the intestinal wall. Histologically, many cases had mononuclear inflammatory cell infiltration of the liver, spleen and small intestine with infiltration less common in other tissues such as the pancreas, heart, kidney and lung. Pulmonary congestion was present in some cases. Parasitic stages were commonly found within intestinal epithelial cells, within macrophages in the lamina propria, and to a lesser extent, in mononuclear cells in extra-intestinal tissues. Both asexual and sexual forms as well as oocysts were present in the intestine (e.g. Rossi *et al.*, 1997) but only asexual forms have been described in mononuclear cells in extra-intestinal tissues such as liver, spleen, lung and kidney (e.g. Ball *et al.*, 1998; Quiroga *et al.*, 2000). The affected birds ranged in body condition from poor (in black siskins, *Carduelis atrata*, Rossi *et al.*, 1997) to fair (in Bali mynahs, *Leucopsar rothschildi*, Partington *et al.*, 1989) and this appeared to be a reflection of the duration of the illness. Harvey *et al.* (1997) recorded *Atoxoplasma* in Indochinese white-rumped shamas (*Copsychus mal.abaricus interpositus*) and found asexual forms in disparate tissues such as skin, feather pulp, brain and bone marrow.

The present investigation was performed in order to record the pathology of coccidial infection in hihi and compare it with records of atoxoplasmosis in other birds and with other coccidial infections in birds in general. The study aimed to improve our

understanding of the pathogenesis of the disease and help in the elucidation of the life cycle of the parasite.

4.2 MATERIALS AND METHODS

4.2.1 Source of material

Five affected hihi obtained from the N.W.C. were examined at Massey University during the period from 1997 to 1999. In addition, a further 7 cases from the pathology archives of the Institute of Veterinary, Animal and Biomedical Sciences, were examined retrospectively.

4.2.2 Necropsy procedure

The procedure followed was essentially the same as that described in Appendix VIII, except that:

a) Blood smears were not routinely performed.
b) The gastrointestinal tract was not routinely stapled onto cardboard for fixing and segments of the tract, rather than the entire tract, were processed.

Sections of all visceral organs were fixed in 10% buffered formalin, routinely processed, embedded in paraffin wax, and cut at 3 μm. The sections were stained with haematoxylin and eosin before examination under a light microscope. During the necropsy of case no. 27561, the intestine was sectioned at approximately 5-10mm intervals throughout its entire length, the tissues processed, and the intestinal wall thickness of the histological tissue measured using Sigma Scan Scientific Measuring System (Jandel Scientific). The measurements were then corrected for shrinkage of tissues during histological processing.

4.3 RESULTS

4.3.1 Case histories

Table 4.1 summarises the epidemiological factors and clinical signs noted in the coccidiosis cases in hihi at the N.W.C.

Table 4.1: Epidemiological factors and clinical signs in affected hihi from the N.W.C.

Case no.	Date of death	Age	Acute death	Other clinical signs
24166	9.3.94	9wks	Y	nil
25108, 3 birds[a]	Jan '95	16days	NR	NR
26375A	9.1.96	30days	Y	nil
26397	12.1.96	32days	N	sick for 2 days
26398	11.1.96	32days	Y	nil
26399	10.1.96	31days	Y	nil
27558	7.1.97	61days	Y	quiet & inactive 5d previously [b]
27561	10.1.97	64days	NR	NR
27721	8-10.3.97	29days	Y	nil
30007	18.2.99	15mnths	Y	[c]

[a] = the three birds were not individually identified

NR = not recorded

Y = yes

N = no

[b] = may have been this bird or an aviary-mate

[c] = one observer reported that the bird appeared to be breathing in a laboured manner shortly before death

All the birds, except one, died between 16 days and 64 days of age. The exception was the hand-reared bird named "Keith" which died at 15 months of age. All the deaths occurred during the months of January, February or March of the relevant year. In three of the 13 cases, clinical signs were noted but these were not consistent. One bird was sick for two days prior to death (specific symptoms not recorded), one bird might have been quiet and inactive five days previously, although this was uncertain and could have been an aviary-mate instead, and one bird was reported to be breathing in a laboured fashion shortly before death, although this observation was disputed by other observers.

4.3.2 Gross findings

Some of the gross findings at necropsy of the N.W.C. hihi affected by coccidiosis are shown in Table 4.2.

Table 4.2: Gross findings in 12 affected hihi from the N.W.C.

Case no.	Swollen abdomen	Thickened intestinal wall	Hepatic spots	Change in liver size
24166	+	+	+	-
25108 3 birds	NR	+	-	-
26375A	NR	+	-	-
26397	NR	+	-	+
26398	NR	+		-
26399	NR	+	-	+
27558	NR	+	-	-
27561	NR	NR	NR	-
27721	NR	-	-	-
30007	NR	-	-	+

NR= not recorded

At necropsy, the most common findings observed were a turgid intestine, an enlarged intestinal diameter and a thickened intestinal wall. In the most severely affected cases the intestine was thickened to twice its normal width over the majority of its length (Fig 4.1). The results of measurements of the thickness of the intestinal wall of case no. 27561 are shown in Figure 4.2.

In case no. 27561, the intestinal thickening was most marked at the proximal jejunum where the intestinal wall measured 2.38 mm. The thickness declined slightly and then increased again to 2.30 mm at approximately the mid to distal jejunum. The colonic wall was much narrower with the most distal segment measuring 0.26 mm.

A swollen abdomen was recorded on one occasion however, the condition of the birds often varied: one was noted to be thin, three in fair condition, one in good condition, and the status of the others was not recorded. A soiled vent was found in one bird. The liver was assessed to be smaller than normal in three birds, and swollen and congested in another. There were numerous small spots on the liver in one case.

4.3.3 Histopathology

Table 4.3 summarises the common histological findings present in the birds examined from the N.W.C.

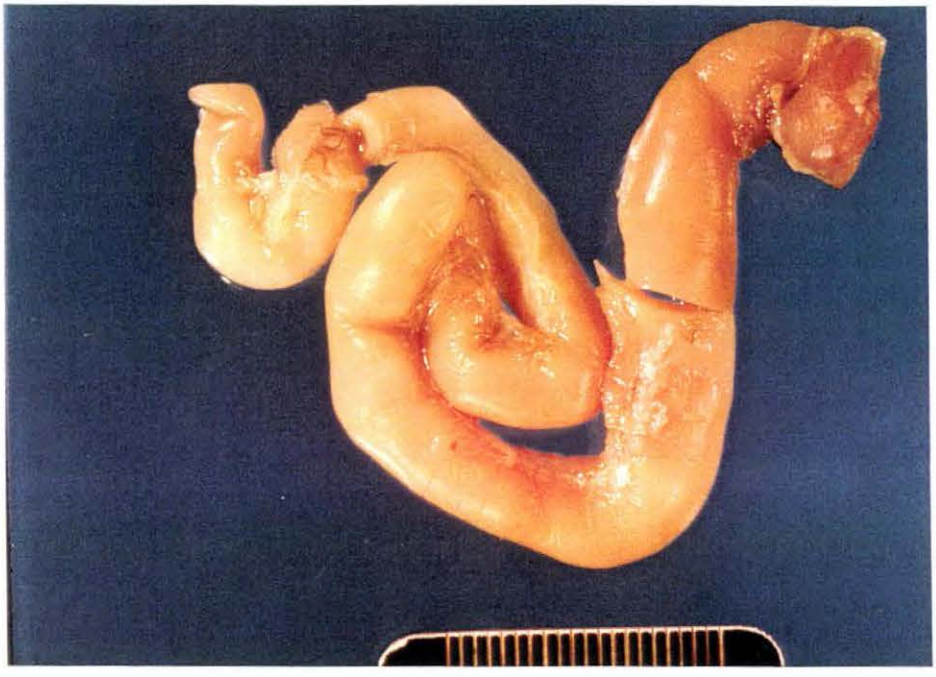


Fig 4.1 Intestine of case no. 27721 showing distended and turgid hihi intestine. This enlargement extends the entire length of the small intestine.

Fig 4.2 Thickness of intestinal wall of case no. 27561

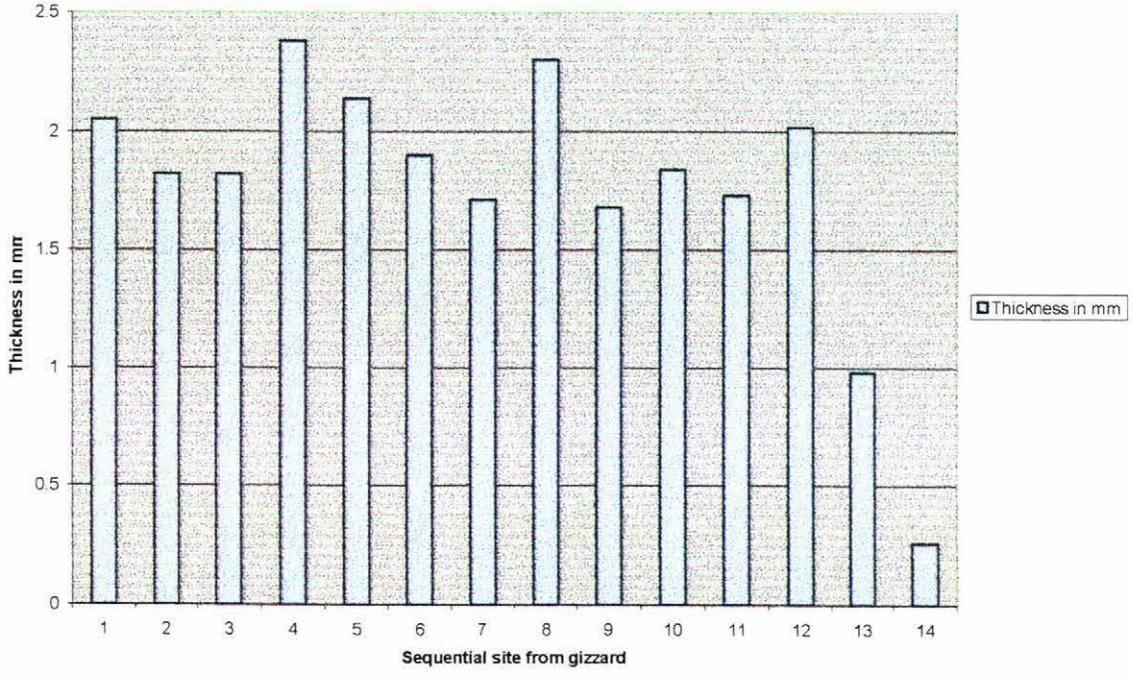


Table 4.3: Histological findings in 12 affected hihi from the N.W.C.

Case no.	Thickening of lamina propria	Coccidia in intestinal wall	Flattening or loss of villi in SI	Destruction of SI epithelial cells	Inflam. foci in liver	Excessive haemosiderin in liver
24166	+	+	+	+	+	+
25108, 3 birds	+	+	+	+	-	-
26375A	+	+	+	+	+	-
26397	+	+	+	+	+	-
26398	+	+	+	+	+	+
26399	+	+	+	+	+	-
27558	+	+	+	+	+	+
27561	+	+	+	+	+	+
27721	+	+	+	+	+	-
30007	+	+	+	+	+	+

All cases had mild to severe thickening of the lamina propria (Fig 4.3), due mainly infiltrating macrophages, many of which contained schizonts and schizozoites.The lamina propria also contained proliferating fibroblasts, moderate numbers of lymphocytes and, in some cases, occasional giant cells. Some schizonts were compact in appearance with the schizozoites in close apposition to one another but with their outlines distinct. Other schizonts contained schizozoites which were slightly separate from one another, and other macrophages contained sections of only one or two schizozoites within the parasitophorous vacuole (Figs 4.4, 4.5, & 4.6). All sexual and asexual stages were present in each bird. The schizozoites were generally banana-shaped in longitudinal section. There appeared to be at least two types of schizonts: those containing up to 6 schizozoites and those containing 10 or more smaller schizozoites. Determination of the number of schizozoites was complicated because of the varying plane of section. Some schizonts were in clusters of 6-8 and others were isolated.

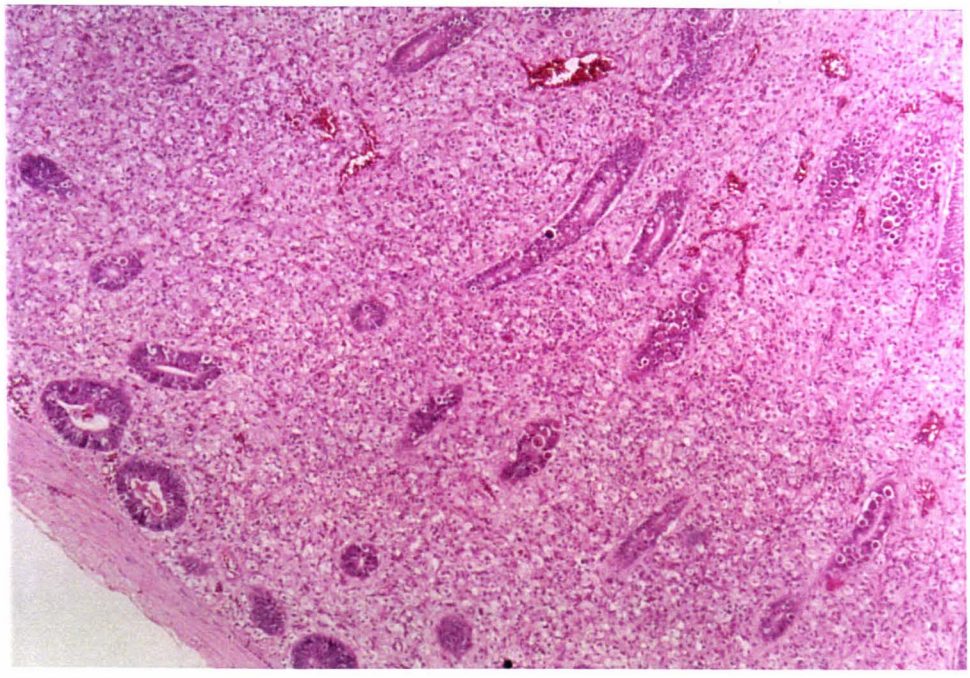


Fig 4.3 The intestine of case no. 27561 illustrating the extreme thickening of the lamina propria caused by macrophage infiltration and fibroplasia. Protozoa are evident in the epithelial glands. H&E x180

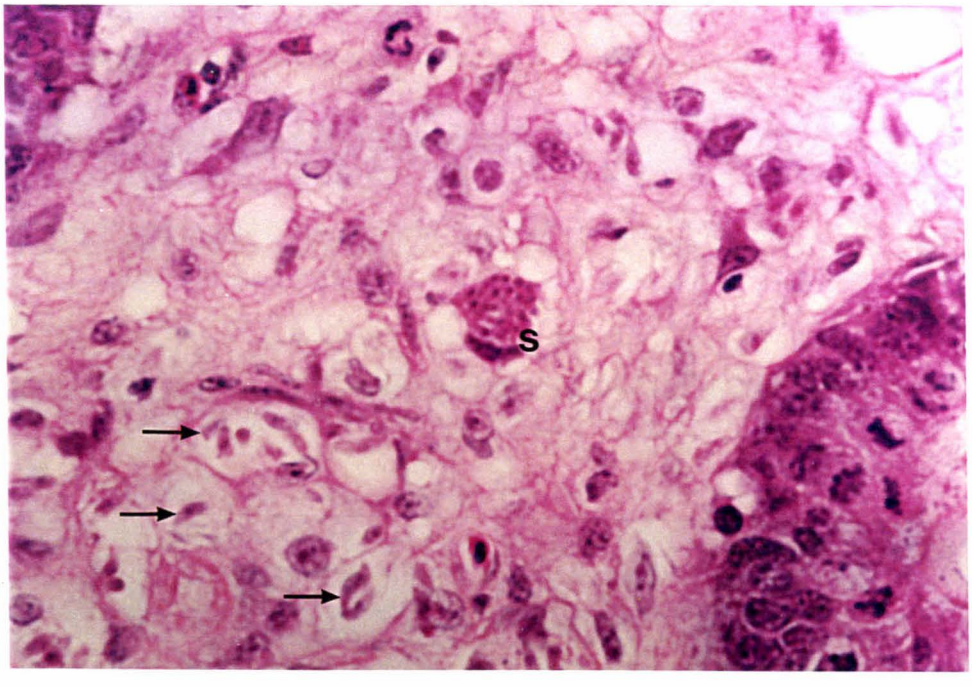


Fig 4.4 The intestine of case no. 26375A showing a schizont in longitudinal section (s) within the lamina propria. There are also several sections through individual intracellular schizozoites (arrows) which are separate from one another. H&E x700

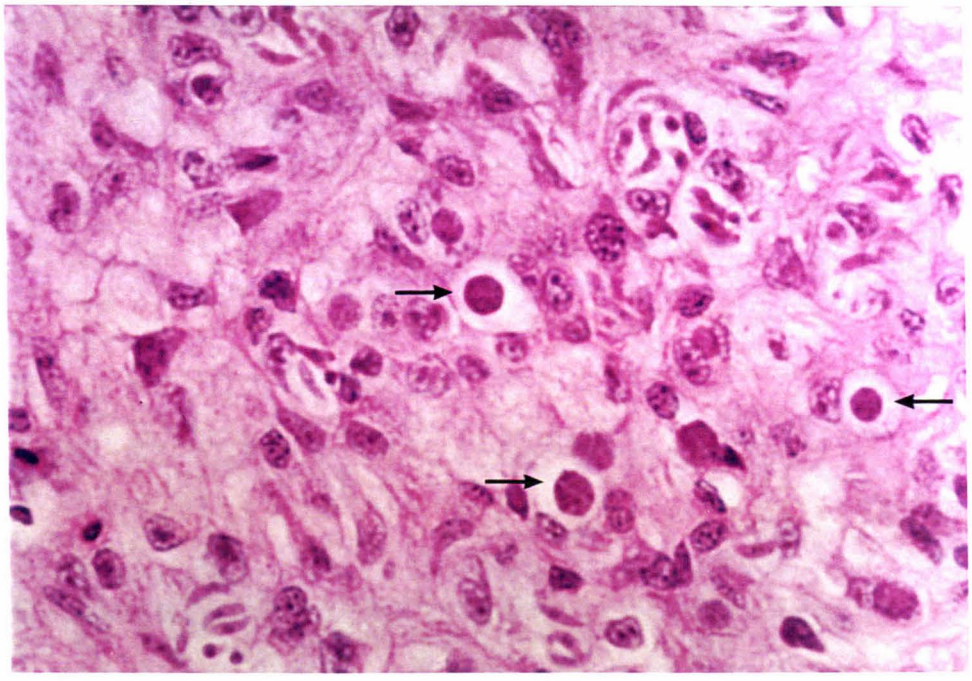


Fig 4.5 Section of case no. 26375A showing several groups of distinct schizozoites in parasitophorous vacuoles as well as several unidentified protozoal stages (arrows) which are probably immature schizonts. H&E x700

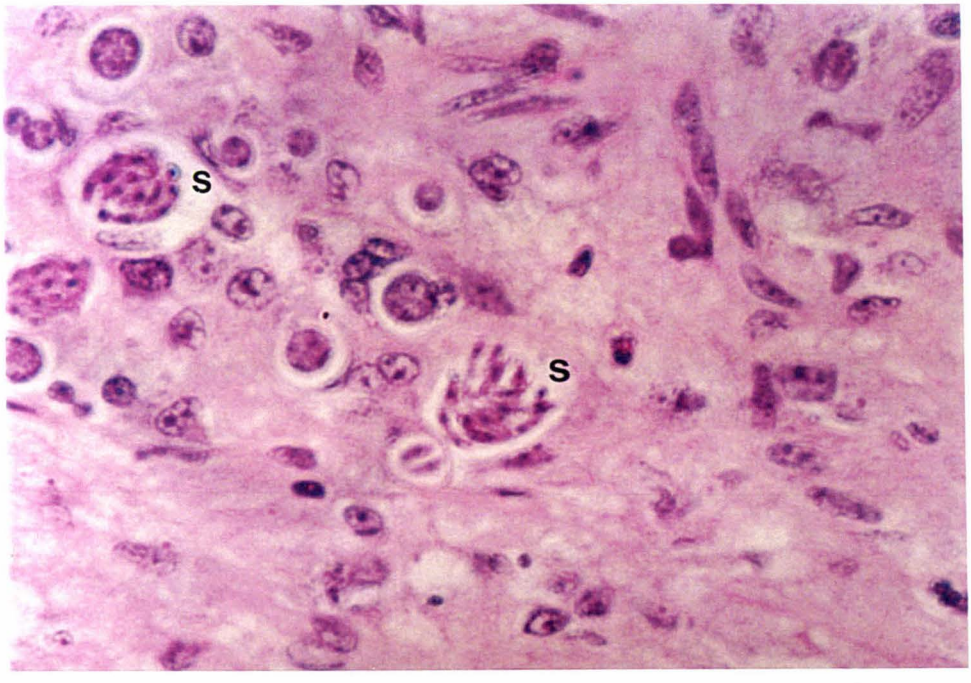


Fig 4.6 Section of intestine of case no. 27561 showing 2 large schizonts (s) containing 10 or more schizozoites in the lamina propria. H&E x700

Also present in the lamina propria were far smaller numbers of coccidial sexual stages and these were often located close to epithelial glands and crypts. It appeared that the epithelial cells of some of these glands and crypts had ruptured and released these stages into the lamina propria. Some protozoal structures, however, were not clear and may have been either early schizonts or gamonts. In addition, occasional oocysts were present (Fig 4.7).

The intestinal villi were often flattened and there was a loss of epithelium, although this change was compounded by autolysis. In most of the affected hihi, there were extremely large numbers of coccidia present in hyperplastic intestinal epithelial cells (Fig 4.8). There were distinct macrogametes with a single central nucleus and peripheral eosinophilic bodies, their precursal macrogametocyte stages, microgametocytes which were similarly sized to the macrogametes or larger, but contained myriad basophilic granules, the microgametes which were small single basophilic bodies, zygotes and oocysts (Fig 4.9). The zygotes resembled macrogametes but contained basophilic structures peripherally and the oocysts had distinct refractile walls around the outside. Many epithelial glands appeared to have no unaffected epithelial cells with all containing a coccidial organism. Other affected glands had ruptured with release of gamonts, zygotes and oocysts into the lumen and into the lamina propria. Some glandular lumina contained very large amounts of necrotic cellular debris, oocysts and invading bacteria, causing marked distension of the glands.

Most cases had scattered, sometimes perivascular, excessive lymphoid foci in the liver (Fig 4.10), and in some there was a more intense focal inflammatory reaction with involvement of heterophils and macrophages, with inflammatory necrosis present in a few cases. In some cases, sexual and asexual coccidial stages were associated with these inflammatory foci (Figs 4.11 & 4.12). The livers of many birds contained excessive haemosiderin within both hepatocytes and Kupffer cells (Fig 4.13).

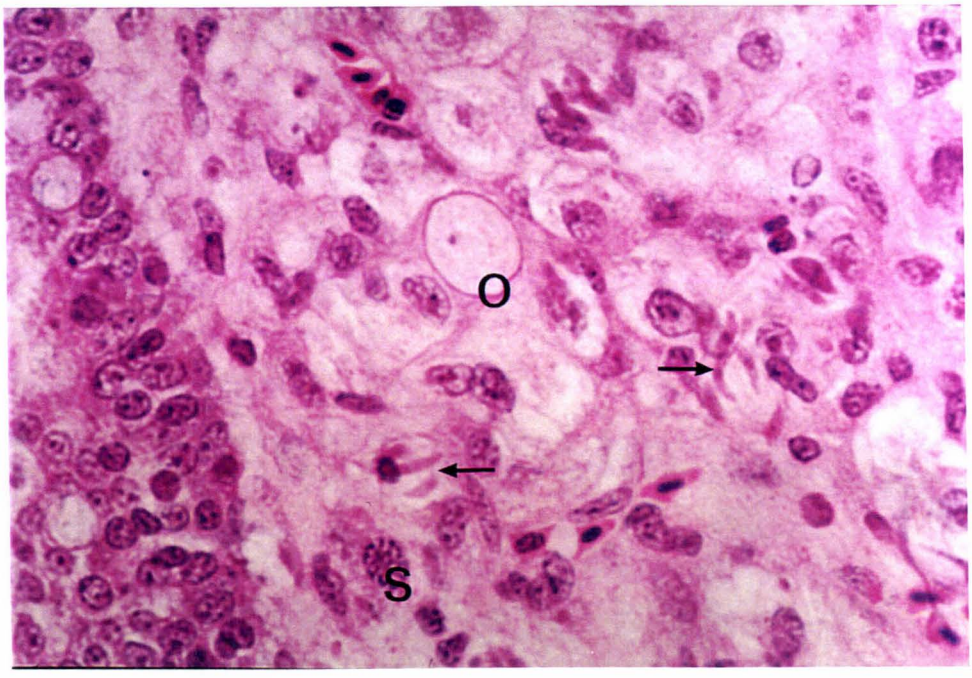


Fig 4.7 The intestine of case no. 26375A showing a large oocyst (O), a schizont in cross section (S) and several schizozoites (arrows) in parasitophorous vacuoles. H&E x700

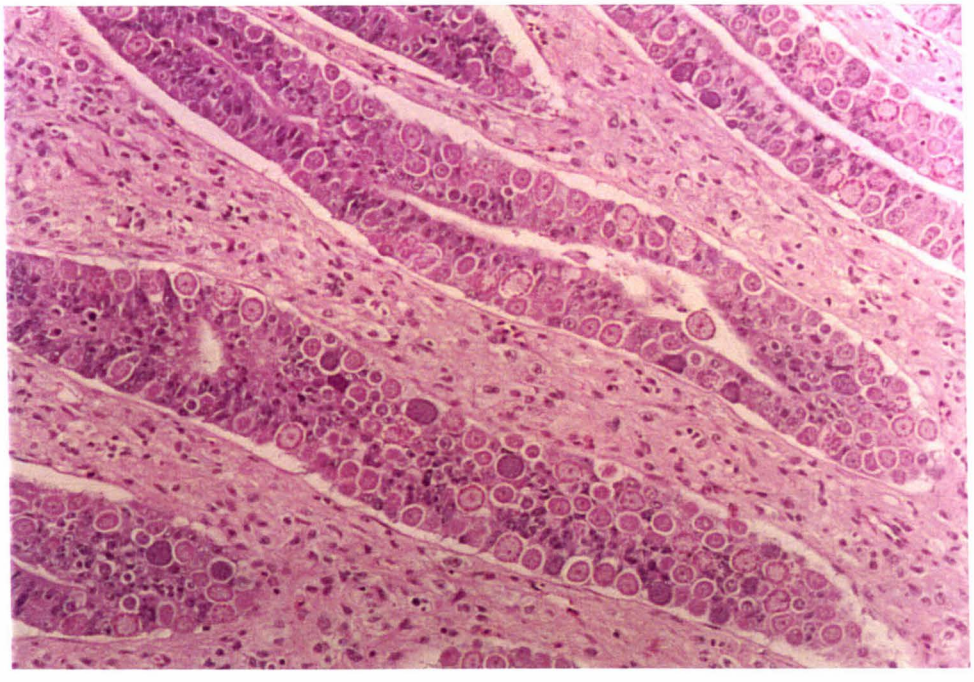


Fig 4.8 The intestine of case no. 27561 showing severe epithelial hyperplasia and the presence of large numbers of sexual coccidial stages within epithelial cells. H&E x270

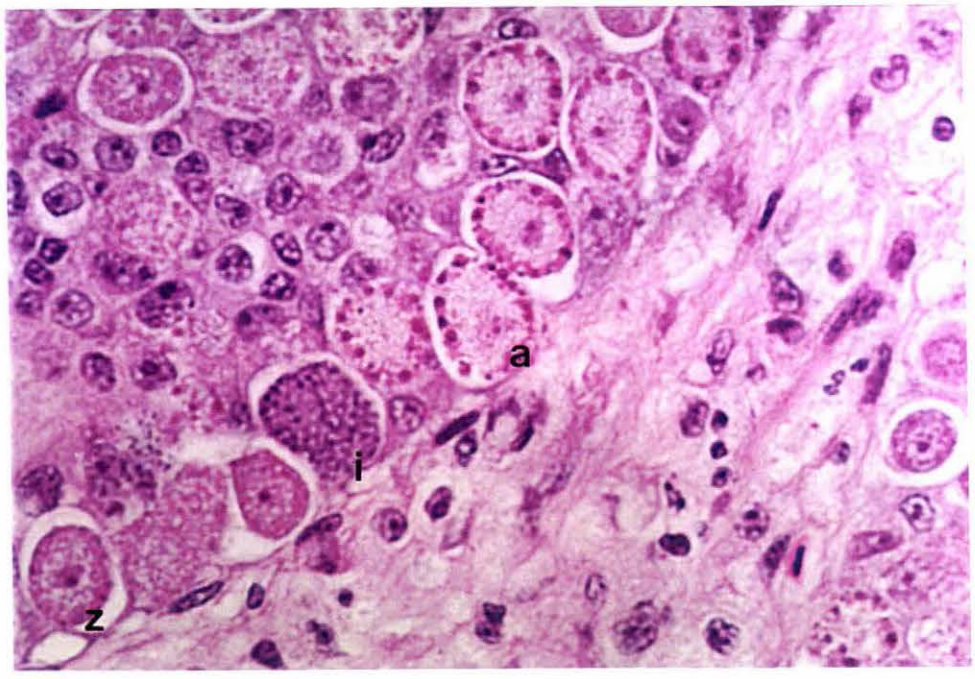


Fig 4.9 The intestine of case no. 27561 showing the base of an epithelial gland and adjacent lamina propria with many macrogametes (a), a microgamete (i), and a possible zygote (z) present in epithilial cells. H&E x 700

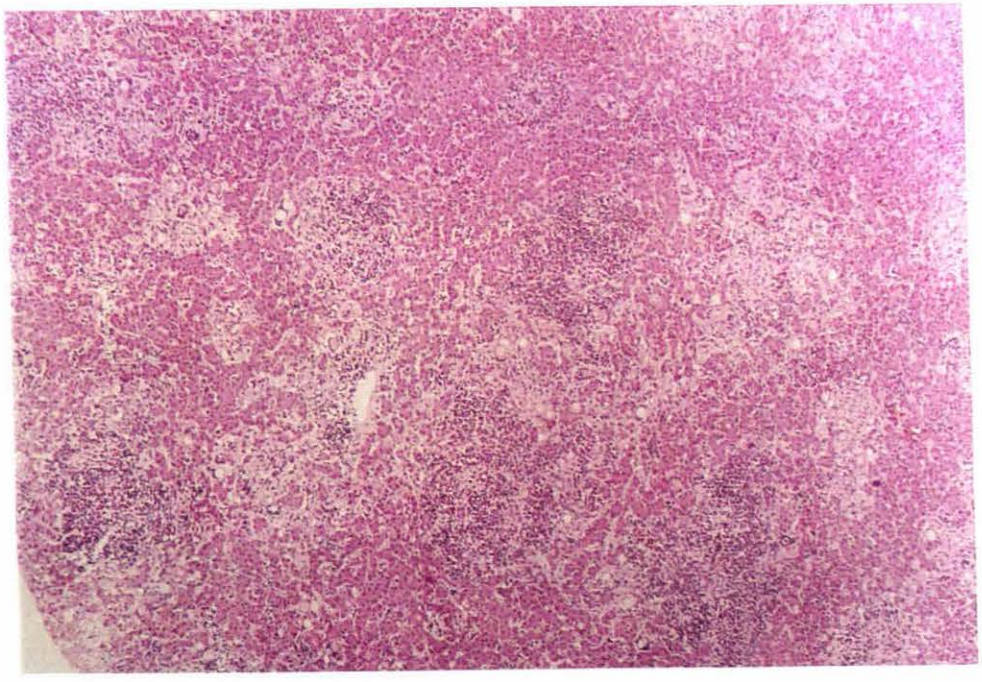


Fig 4.10 Section of liver from case no. 26375A showing scattered multifocal areas of mixed inflammatory cell infiltration. H&E x100

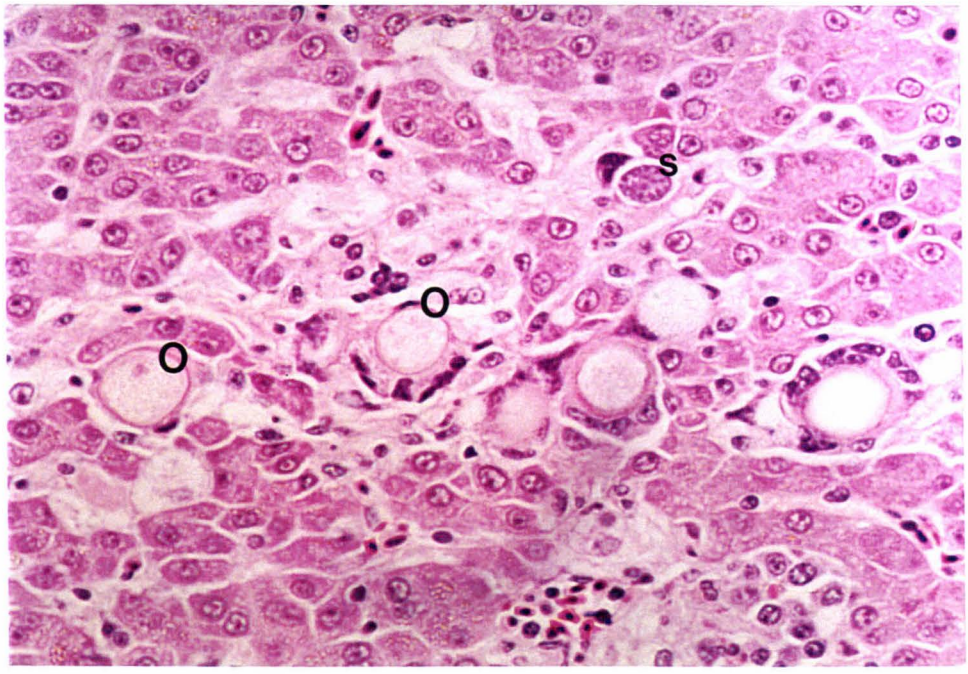


Fig 4.11 Section of liver from case no. 26375A showing several distinct oocysts (o) with complete oocyst walls and a schizont (s) surrounded by its parasitophorous vacuole within a macrophage. H&E x 540

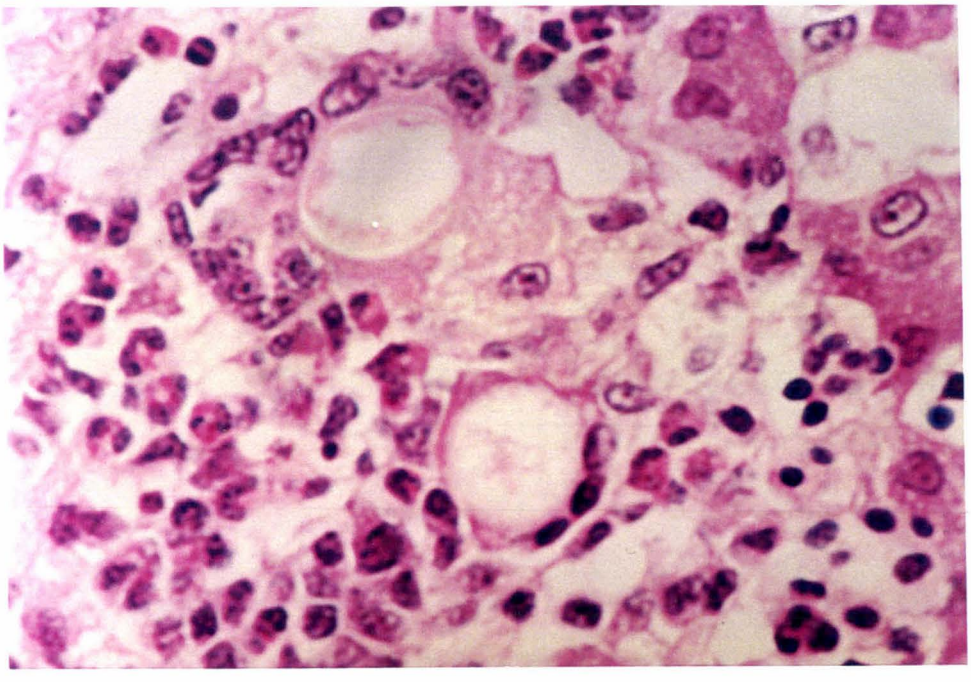


Fig 4.12 High power section from case no. 26375A showing two oocysts within macrophages. H&E x1070

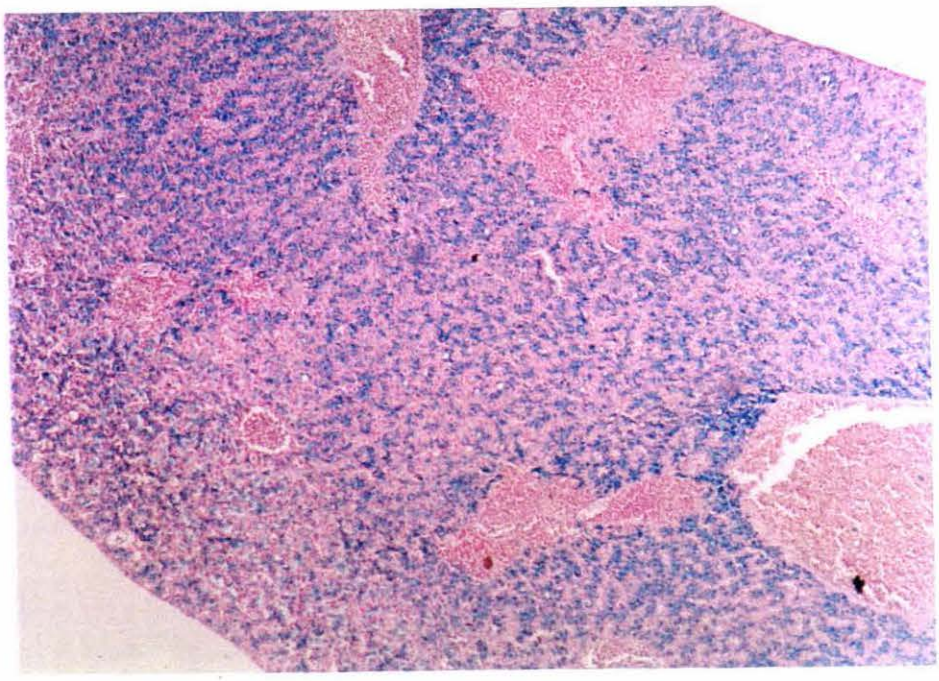


Fig 4.13 Section of liver from case no. 27561 showing severe deposition of haemosiderin. Perl's stain x70

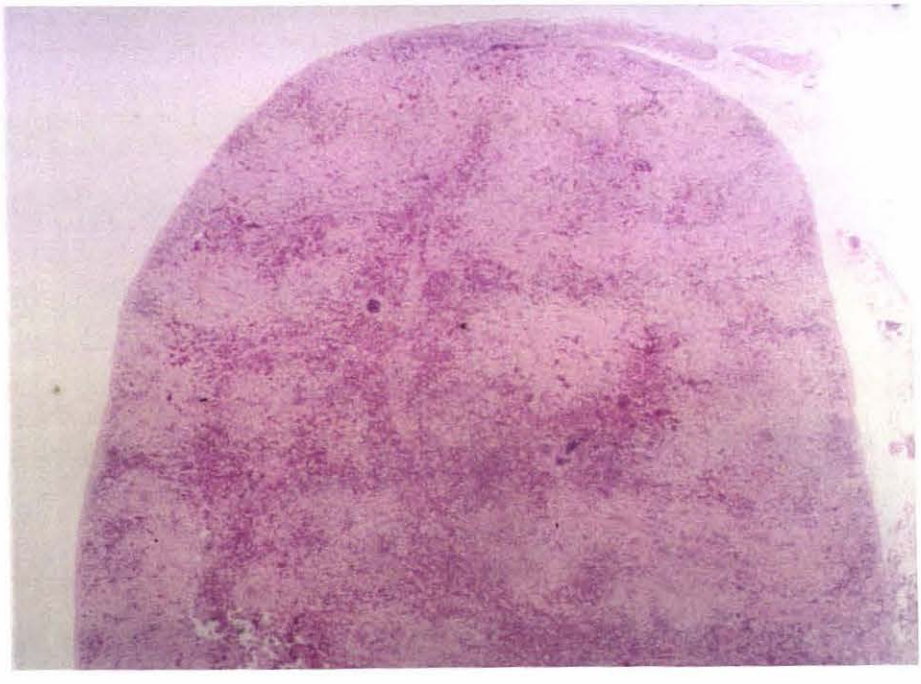


Fig 4.14 Low power view of spleen from case no. 26375A showing severe proliferation of histiocytic cells. H&E x27

In some cases there was proliferation of histiocytic cells in the spleen (Fig 4.14). In others there was some splenic vascular thrombosis, and in two cases there was necrosis in the spleen, which took the form of small foci of recent inflammatory necrosis in one bird, and occasional necrotic lymphoid cells in the other (lympholysis). The splenic lesions were sometimes associated with schizonts which were obvious as distinct bundles of schizozoites in parasitophorous vacuoles of macrophages (Fig 4.15). In one case these vacuoles contained small dense eosinophilic bodies which resembled protozoal remnants.

In two cases there were occasional schizont-like structures in the kidney tissue but these could not be confirmed. In one of these cases there were structures which resembled degenerative protozoa in mononuclear cells in the renal interstitium and in the other case there were occasional coccidia-like structures in renal capillary endothelial cells. In case no. 27721 there was a schizont in a blood vessel within the kidney (Fig 4.16).

Table 4.4 shows the location of the different coccidial life cycle stages found in the hihi cases examined from the N.W.C.

Table 4.4: Presence and location of coccidial organisms in 12 affected hihi from the N.W.C.

Case no.	Intestinal epithelium	Lamina propria	Liver	Kidney	Spleen
24166	S, O	AS	S, O	AS[a]	nil
25108, 3 birds	S, O	AS, S	O	nil	nil
26375A	S,	AS, S	AS, S, O	nil	AS, S
26397	S, O	AS	S, O	nil	AS
26398	S, O	AS, S, O,	AS, S, O	nil	AS
26399	S, O	AS, O	S, O	NE	AS
27558	S	AS, S	nil	nil	NE
27561	S, O	AS, S	nil	nil	nil
27721	S, O	AS, O	AS	AS[a]	NE
30007[b]	S	AS, S	nil	nil	AS, S[c], O

AS = asexual stages
S = sexual stages
O = oocysts

NE = not examined
[a] = structures could not be confirmed as protozoa
[b] = tissues moderately autolysed
[c] = uncertainty over classification of protozoal forms
Note: It was difficult to differentiate early sexual stages from early asexual stages in the lamina propria.

All birds had coccidial stages in the intestine and 8/10 cases (case no. 25108 is referred to as one case because the three birds were not individually distinguished) had stages in extra-intestinal tissues. In addition to asexual stages (schizonts and schizozoites) being present in the lamina propria, they were seen in the spleen of 5/10 cases and in the liver of 3/10 cases. In all tissues the schizonts were located in parasitophorous vacuoles within macrophages where they took the form of bundles of banana-shaped schizozoites. Sexual stages (macrogametes, microgametes and their developmental stages) were present in intestinal epithelial cells of all cases, in the lamina propria of 6/10, in the liver of 5/10, and in the spleen of one bird. In these extra-intestinal tissues the coccidia were again present in macrophages (including the Kupffer cells of the liver). In the lamina propria the gamonts were usually in macrophages but in some instances might also have been extracellular. Oocysts were present in the intestinal epithelium of 7/10 cases, the liver of 6/10, the intestinal lamina propria of 3/10, and the spleen of one case.

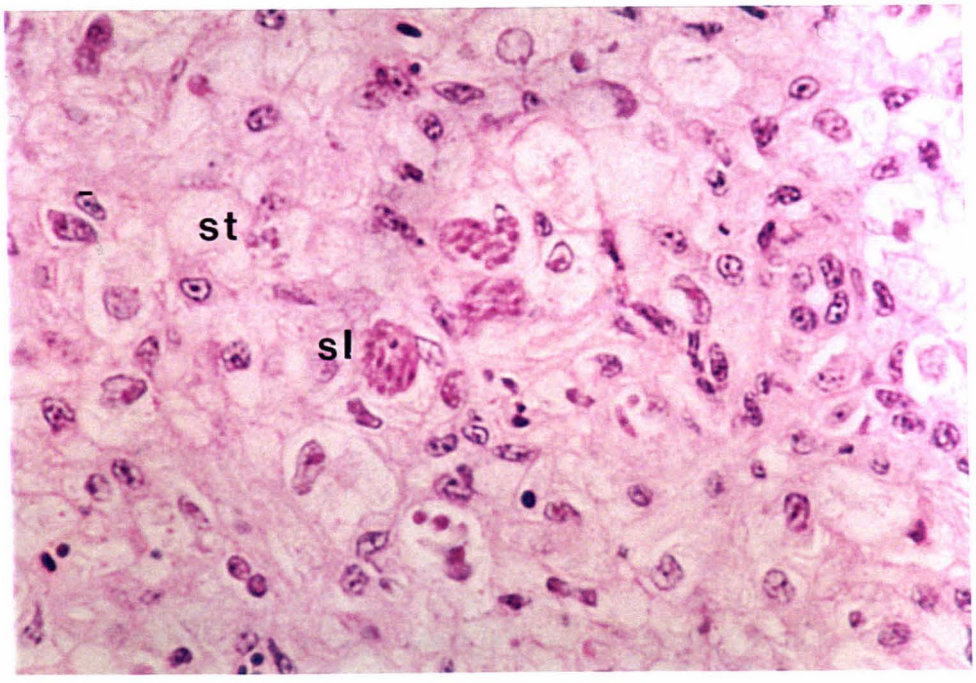


Fig 4.15 Section of spleen from case no. 26375A showing several schizonts within macrophages, both in longitudinal section (SL) and in transverse section (ST). H&E x700

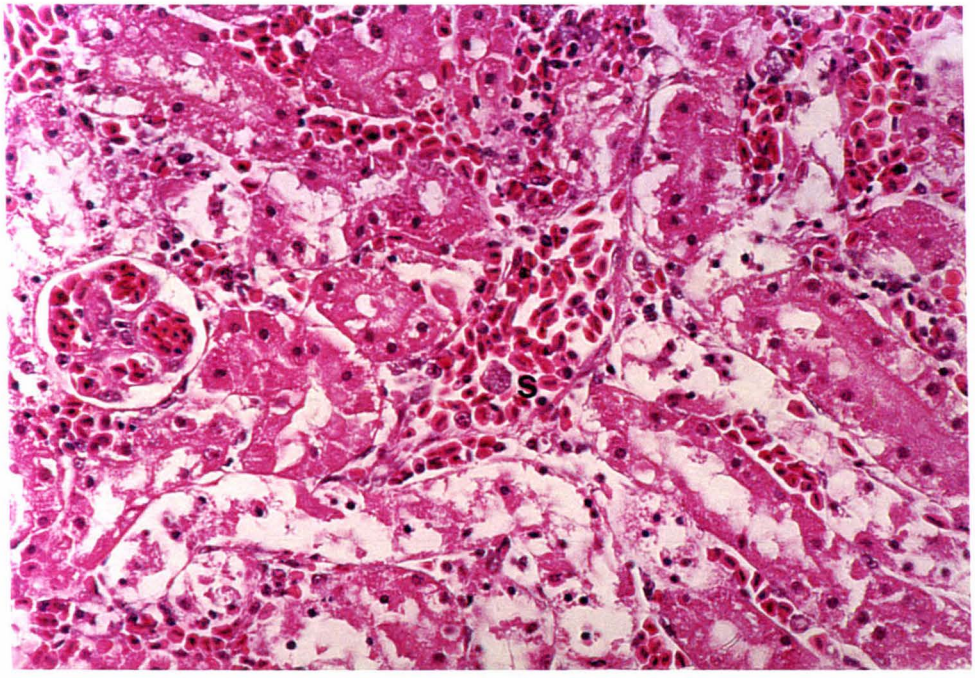


Fig 4.16 Section of kidney from case no. 27721 showing a schizont (s) within a blood vessel in the renal interstitium. H&E x350

4.4 DISCUSSION.

The major gross necropsy findings in the hihi in this study were distended loops of intestine due to an enlarged intestinal diameter and a thickened intestinal wall.

In contrast to the findings reported in many other species (Helman *et al*, 1984; Partington *et al*, 1989; Harvey *et al*, 1997) neither hepatomegaly nor splenomegaly were a consistent finding in hihi although a reduction of liver size was recorded on three occasions and a slightly swollen and congested liver on one occasion. The fact that the birds were, in most instances, individually submitted and that there was a variation in age, meant comparison with other cases was difficult. Subjective assessments such as liver and spleen size, are likely to have a high degree of error both within and between operators.

Because of the retrospective nature of some of the pathological data presented here, there was no set protocol followed for the necropsies. Although the veterinarians involved all performed necropsies in a similar fashion, the procedures used were not identical, and for this reason, the information recorded cannot all be compared accurately e.g. one veterinarian requested that the bodies be submitted already fixed in formalin whereas others preferred them to be sent fresh if practical; prior fixation would have masked some gross features although improved histological preparation.

In most cases, the birds died per-acutely with few premonitory signs. If signs of illness were evident, these were generally non-specific signs such as fluffed-up plumage and inactivity. Although a swollen abdomen was only recorded on one occasion, it is probable that it was in fact present in more cases because severe thickening of the intestinal wall was frequently recorded. The swollen abdomen could, therefore, be a useful, more specific clinical sign.

Body condition of the affected birds was often not mentioned in the necropsy records. This is partly because of the aforementioned reasons (lack of comparisons, multiplicity of

persons performing the necropsy and the subjective nature of the assessment) and also because hihi are very small birds (adult weight 25-50g) and do not normally carry noticeable fat stores.

The outstanding histological feature of the cases in the current series was the intestinal pathology. There was, in most cases, a spectacular invasion of the lamina propria and intestinal epithelial cells with coccidial stages, along with, frequently, an extreme thickening of the lamina propria due to macrophage infiltration and proliferation of asexual stages of the organism. This degree of intestinal pathology was not present in the cases of atoxoplasmosis (or systemic coccidiosis) described by Poelma *et al.* (1971), Helman *et al.* (1984), Partington *et al.* (1989), Cooper *et al.* (1989), McNamee *et al.* (1995), Baker *et al.* (1996), Harvey *et al.* (1997), Rossi *et al.* (1997), Quiroga *et al.* (2000). In fact, there was no intestinal pathology noted in *Lankesterella/Atoxoplasma*-infections seen in a variety of bird species from the Netherlands (Poelma *et al.*, 1971), zebra finches (*Poephila guttata castanotis*) (Helman *et al.*, 1984) (although this infection was identified only as a "systemic protozoal disease"), a bullfinch (*Pyrrhula pyrrhula*) (McNamee *et al.*, 1995), or in a northern cardinal (*Cardinalis cardinalis*) (Baker *et al.*, 1996), although this latter case was also not identified at the time as atoxoplasmosis, but rather as a *Lankesterella*-like organism. In Bali mynahs there was a diffuse infiltration of the lamina propria by a mixed inflammatory cell population, but no intracellular organisms in inflammatory cells of the intestine (Partington *et al.*, 1989). However, in the black siskins with systemic coccidiosis, examined by Rossi *et al.* (1997), the small intestinal wall was thickened (four to five times greater than normal) and dilated, there was a heavy transmural lympho-monocytic enteritis, the duodenal and jejunal epithelium was badly damaged by various coccidial stages (gamonts, oocysts and several schizonts), and zoites were present in the cytoplasm of the granular cells, macrophages and lymphocytes in the intestine. Rossi *et al.* (1997) suggested that the coccidia in the black siskins was an *Isospora* species. In the Indochinese white-rumped shamas described by Harvey *et al.* (1997), there were myriad coccidia seen in epithelial cells, especially in the duodenum, of two birds. Three of 14 greenfinches (*Carduelis chloris*) examined by Cooper *et al.* (1989), showed a moderate to

marked cellular infiltrate, mainly mononuclear, of the lamina propria of the small intestine but no coccidial organisms were reported in the intestine. In a canary (*Serinus canarius*) described by Quiroga *et al.* (2000), there was necrosis and marked cellular infiltration of mainly mononuclear cells, in the lamina propria of the small intestine and intracytoplasmic merozoites (schizozoites) were identified ultrastructurally in the infiltrating macrophages.

In the affected hihi, hepatic lesions, and in some cases, splenic lesions, were also an important feature. Most cases had excessive lymphoid foci in the liver, and in some there was a more intense inflammatory reaction with involvement of heterophils and macrophages, with focal necrosis present in a few cases. Many livers contained excessive haemosiderin in hepatocytes and Kupffer cells. In some of the present cases there was proliferation of histiocytic cells in the spleen but in others splenic lesions were variable. Some showed splenic vascular thrombosis, in one case there was splenic focal inflammatory necrosis, and in another there were occasional necrotic lymphoid cells.

There is some similarity between the hepatic and splenic lesions seen in the hihi and those described in other birds diagnosed with atoxoplasmosis or similar isosporan infections. In the bird species infected with *Lankesterella/Atoxoplasma* described by Poelma *et al.* (1971), there was inflammation and necrosis in the liver, and, in addition, many blood vessels in the liver showed signs of degeneration, lesions which the authors suggested may have been caused by a toxin rather than be associated with the coccidial infection. In the zebra finches described by Helman *et al.* (1984), there was a marked splenomegaly in 5 of 6 birds and a hepatomegaly in two of the birds, with necrosis and a granulomatous inflammation in the liver and spleen. There was hepatosplenomegaly and haemochromatosis in the Bali mynahs described by Partington *et al.* (1989). In 6 of 14 greenfinches examined by Cooper *et al.* (1989), there was a predominantly mononuclear perivascular inflammatory infiltrate in the liver and lymphoid hyperplasia in the spleen. In the bullfinch with atoxoplasmosis, described by McNamee *et al.* (1995), there was hepatosplenomegaly with congestion and perivascular inflammation in the liver and granulomas in the spleen. There was no mention of hepatic or splenic pathology in the

northern cardinal described by Baker *et al.* (1996). In the Indochinese white rumped shamas described by Harvey *et al.* (1997), there was hepatosplenomegaly with infiltrations of epithelioid macrophages and multinucleate cells while in the black siskins described by Rossi *et al.* (1997), there was hepatomegaly and a slight splenomegaly with lymphomonocytic infiltrations in the liver and activation of lymphoid follicles in the spleen. In the canary described by Quiroga *et al.* (2000), there was necrosis and marked cellular infiltration of mainly mononuclear cells, in the liver and spleen.

In the hihi studied here, numerous asexual coccidial stages were found in mononuclear cells in the intestinal lamina propria, and to a lesser extent, in mononuclear cells in extraintestinal tissues, including liver, spleen and, in one case, the kidney. Sexual stages were found in intestinal epithelial cells and in the lamina propria. Differentiation of sexual stages from asexual stages in the lamina propria was difficult with light microscopy and it is possible that some of the organisms identified as sexual stages were early asexual stages. Electron microscopy might assist identifying these stages more conclusively. Sexual stages were also occasionally found in mononuclear cells in the liver and spleen. Oocysts were found in the intestinal epithelium, lamina propria, liver, and in one case, in the spleen.

In the literature already cited, asexual protozoal stages were found in extra-intestinal tissues in bullfinches, a variety of bird species in the Netherlands, northern cardinal (schizonts in the lung), black siskins, Indochinese white rumped shamas, and a canary. In black siskins, asexual protozoal stages were also found in granular and mononuclear cells in the intestine and in intestinal epithelial cells (Rossi *et al.*, 1997). In the canary, asexual stages were also found in mononuclear cells in the intestine (Quiroga *et al.*, 2000). Sexual protozoal stages were found in the lung in a northern cardinal (Baker *et al.*, 1996), and in the intestinal epithelium of black siskins and Indochinese white rumped shamas. Oocysts were also found in the lung of a northern cardinal (sporulated oocysts) and in the intestinal epithelium of black siskins and a canary. Unclassified protozoal stages were found in extra-intestinal tissues in Bali mynahs, greenfinches and zebra finches. Thus, none of the case histories or descriptions of atoxoplasmosis or *Atoxoplasma*-like infections which have

been reported to date, have exactly the same distribution of their protozoal stages as that which has been found in the hihi.

Because the hihi cases described here, and the cases in other species which have been referred to in the literature, died at some unidentifiable point during the progress of an incompletely understood infection, it is very difficult to interpret and make valid comparisons. In order to ascertain the exact life cycle/developmental stages and the natural progression of infection, it would be necessary to necropsy birds at known time intervals after infection. In the cases described here, the time at which infection occurred is not known.

According to Levine's definition of *Atoxoplasma* (1982), asexual stages should be found in intestinal and extra-intestinal tissues (including blood) and sexual stages in intestinal cells only. This study of the hihi cases has found sexual stages in extra-intestinal tissue also. However, findings of this type have not deterred other workers from classifying similar organisms as atoxoplasms. Baker *et al.* (1996) found gamonts in the lung in a northern cardinal. The present author did not find schizonts in intestinal epithelial cells as Rossi *et al.* (1997) did in black siskins. Neither did the present study find zoites in granular cells as Rossi *et al.* (1997) also did.

Although there are some common aspects in the hepatic and splenic pathology of published atoxoplasma cases and the hihi cases, the intestinal lesions are markedly different from those described in other species. There are also some similarities in the tissue location of the different coccidial life cycle stages but there is no conclusive pattern in any of the species studied. Therefore, based on the gross necropsy findings and the histopathology, classification of the hihi coccidial infection remains inconclusive.

CHAPTER FIVE

GENERAL DISCUSSION

This thesis records the first detailed study of a coccidial infection which has had a marked deleterious effect on the captive population of hihi at the National Wildlife Centre (N.W.C.). Because of a high mortality rate in the juvenile birds, few were reaching a stage where they were available for movement to the wild populations. The disease had proved difficult to contain and the population was unable to grow, thereby hindering the captive breeding programme and species management.

The initial pathological studies of hihi which died from this infection, indicated the involvement of an isosporan-type coccidian with extra-intestinal stages and so the possibility of infection with *Atoxoplasma* species was proposed. Further studies were required in order to learn more about the life cycle and epidemiology of this parasite; however, the research techniques were limited because of impossibility of carrying out experimental procedures or elective necropsies on the birds themselves, due to their high individual conservation value.

The study described here comprised three, interrelated parts. The first, aimed to gather as much information as possible about the infective stage of the parasite by looking at oocyst shedding and the morphology of sporulated oocysts. The second part was intended to look at the infectivity and species-specificity of the organism by conducting transmission experiments where oocysts were inoculated into birds of the same and different species and signs of subsequent infection were investigated. The third stage was a detailed study of the pathology of the infection in the hihi at the N.W.C. using both current and retrospective necropsy cases.

The hihi chicks in each of the two breeding seasons studied, had at least two periods of oocyst shedding: one before fledging while still in the nest and one or more episodes post-

fledging with a high shedding period between 6.5-8 weeks of age. The earliest recorded excretion of oocysts in the chicks was at 9 days of age. Oocyst shedding in the adults was less common and less consistent with no patterns apparent. In all age groups, production of oocysts appeared to be eliminated, at least temporarily, by toltrazuril treatment. However, it sometimes took up to 7 days after the beginning of a 5 day course of toltrazuril for oocyst numbers to decline to zero, and numbers often rose again very quickly. In one instance oocyst numbers were positive again one day after treatment had eliminated shedding. These results indicate that although the toltrazuril treatment was effective in eliminating oocyst production, the drug does not kill all stages of coccidia present within the bird. It therefore seems likely that the extra-intestinal stages of the parasite were not being eliminated, thereby providing a source from which further intestinal infections develop.

No juveniles died of coccidiosis during the research period when oocyst counts were being closely monitored and treatment initiated when counts were elevated. This adds further support to the assumption that the toltrazuril is partially effective in controlling the disease. However, this method of control does come at a cost. Almost daily faecal analyses would be necessary to effectively monitor the disease and this is highly labour intensive and time consuming.

The adult bird "Keith" was unusual in that it had frequent periods of shedding of high numbers throughout 1998 and in early 1999 and eventually died of a coccidial infection, the only adult hihi on record to do so. This bird was hand-reared from nestling stage and so did not have the exposure to other hihi that other birds have, both in the wild and at the N.W.C.. It is thought that this lack of exposure to conspecifics hindered the development of a strong natural immunity to coccidial infection rendering the bird susceptible in adult-hood. Although the bird received many treatments with toltrazuril, this product is claimed not to interfere with the development of immunity (Baycox Product Information, Bayer), and many of the N.W.C.-hatched hihi have received numerous treatments with the same drug and become less susceptible to infection as they matured. This is, therefore,

consistent with the possibility that toltrazuril does not prevent the development of immunity to coccidiosis.

Oocysts were also found in faeces shed by hihi from Tiritiri Matangi and Mokoia Islands. After sporulation, the oocysts were able to be allocated into two morphologically different groups: Type A which comprised oocysts of a subspherical shape, and Type B which comprised ellipsoid oocysts. Oocysts from the N.W.C. hihi were all of the subspherical type, 40% of oocysts recovered from Tiritiri Matangi were subspherical and 60% ellipsoid, and 37.5% of oocysts recovered from Mokoia Island were subspherical and 37.5% ellipsoid (the other 25% of oocysts recovered from Mokoia were two larger oocysts which were not classified as Type A or Type B). The sporulation of oocysts from both Tiritiri Matangi and Mokoia Islands occurred without difficulty whereas those from the N.W.C. resisted most of the methods attempted in the laboratory. There were no detectable differences in morphology between oocysts from the N.W.C. and those from the other two locations, therefore it seems that the difference in sporulation cannot be explained by them being different species, but instead might be due to unknown or unrecognized features associated with the managed, captive environment at the N.W.C.. While there is no evidence of any efficacy on unsporulated or sporulated coccidial oocysts by toltrazuril in poultry (Baycox Product Information, Bayer), there is the possibility that using toltrazuril at the N.W.C. might have affected sporulation of the hihi oocysts on at least some occasions.

The study has confirmed the risk seasons and the at-risk age groups. As well, further useful information was gathered regarding the effectiveness of toltrazuril treatment. Other issues which were considered but not resolved included the effect of drugs such as itraconazole on oocyst shedding, diurnal periodicity of oocyst shedding, and the effect of the breeding season on bird susceptibility. The source of infection is also still not clear and although none of the data gathered in this study supports the idea that the adults harbour sub-clinical infections and pass the parasite onto the chicks, this cannot be discounted. More likely is the possibility that the infection is maintained in chicks and juveniles and the

oocysts are remaining viable in the environment for long periods of time. There is a need for further work on this issue, specifically looking at oocyst survival times.

The starling (*Sturnus vulgaris*) and hihi sections of the transmission studies were inconclusive because the proposed experimental procedures could not be completed due to unavailability of sporulated oocysts. In the zebra finch (*Poephila guttata castanotis*) transmission study, infection of the finches with the coccidia from the hihi did not occur. Although this provides some evidence that these coccidia are host specific it is far from conclusive and further transmission studies are needed. Zebra finches are not a species which would be expected to share parasitic susceptibility with hihi because no close phylogenetic relationship exists between them. They are also not a species of importance in relation to passage of infection to the captive hihi population from wild birds as they are not present in or around the N.W.C. aviaries. Zebra finches were chosen as the species for use in this transmission experiment because of their availability and the ease with which they can be kept in captivity. The methods used could be drawn upon for future studies. More relevant bird species to use in order to determine host specificity would be the bellbird (*Anthornis melanura*) or tui (*Prosthemadera novaeseelandiae*) (both belong to same family, *Meliphagidae*, as the hihi, but are also protected species), starlings or sparrows (*Passer domesticus domesticus*) as both are abundant in the area outside the aviaries.

Most affected hihi died per-acutely with few premonitory signs. If signs were present they were generally non-specific such as fluffed-up plumage and lethargy. On gross pathology, the main feature was distended loops of intestine due to an enlarged intestinal diameter and a thickened intestinal wall. This finding would be easily overlooked at necropsy by pathologists unfamiliar with the size and thickness of the hihi intestinal tract which is normally shorter than that of other passerines of comparable size.

Histopathology revealed remarkable intestinal lesions with severe invasion of the lamina propria and intestinal epithelial cells with coccidial stages and an extreme thickening of the

lamina propria due to macrophage infiltration and proliferation of asexual coccidial stages. Lesions were also commonly found in the liver and these consisted of excessive lymphoid foci, and, in some cases, more intense inflammatory reactions with heterophils and macrophages. Excessive haemosiderin was also often present in the liver. This is a non-specific change which is often associated with concurrent malignant and infectious disease (Cork *et al.*, 1995).

In affected hihi, asexual coccidial stages were present in macrophages in the lamina propria and, to a lesser degree, in mononuclear cells in the liver, spleen, and in one case, in the kidney. Sexual coccidial forms were present in intestinal epithelial cells and, in smaller numbers in the lamina propria. Also, on occasion, sexual forms were found in mononuclear cells in the liver and spleen. Oocysts were present in the intestinal epithelium, the lamina propria, and, on one occasion, in the liver. This pattern of pathology and of location of coccidial stages is different to all *Atoxoplasma* or *Atoxoplasma*-like cases in birds previously described in the literature (Helman *et al.*, 1984; Cooper *et al.*, 1989; Partington *et al.*, 1989; Panigahy and Senne, 1991; McNamee *et al.*, 1995; Norton *et al.*, 1995; Giacoma *et al.*, 1997; Martinez and Muñoz, 1998; Ball *et al.*, 1998; Quiroga *et al.*, 2000). Although there are many similarities, particularly in hepatic and splenic lesions and, to some extent, in the location of the coccidial stages, the intestinal lesions in the hihi are much more florid than those previously described. There is, however, no consistent pattern of lesions or location of coccidial stages in documented *Atoxoplasma* and *Atoxoplasma*-like infections in birds (e.g. Helman *et al.*, 1984; Partington *et al.*, 1989). Because of this lack of consistency, no conclusions can be drawn from the pathological findings on the classification of the coccidial agent in the hihi.

There can be little doubt that the protozoal agent in the hihi is an isosporan-type coccidian. The exact taxonomic classification cannot be ascertained with any great certainty as a result of this present research. Electron microscopy of organisms is therefore necessary to enable a name to be given to the parasite. Examination of blood samples for parasitic stages in leucocytes could also help to provide more information about the life

cycle. This was not performed in the current study because of the requirement to limit stress in the birds; however, it could be a valuable exercise in future screening and transmission studies.

On the basis of the information acquired in this research, there is little additional justification for placing the organism firmly in the Genus *Atoxoplasma.* It appears just as likely that it belongs to another genus or family altogether, such as the Genus *Isospora*, because research in the last 30 years has shown that some isosporan organisms have extra-intestinal stages e.g. some feline and canine *Isospora* species (Dubey and Frenkel, 1972; Dubey, 1975, 1978, 1979). The taxonomic classification of the organism is important because it enables assumptions to be made about the life cycle and characteristics of the parasite. However, given the uncertainties remaining about the identification and the life cycles of species of *Atoxoplasma*, there seems little to be gained at this point in placing the organism that is the subject of this study in that genus.

In order to better understand the biology of this disease there is much work which remains to be done. It is important to know more about the parasitic and disease status of the hihi population on Little Barrier Island (L.B.I.), the only self-sustaining population of this species. Although oocysts were found in faeces from a hihi captured from L.B.I. (J. Pauli, pers. comm.), we have no knowledge of hihi disease status as necropsies have not been carried out on birds from this population. It is also important to determine the host specificity of the organism. Although the present study has shown no evidence of transmission to finches the question of transmission to other species is by no means settled. Now that detailed morphological descriptions of hihi coccidia have been carried out, widespread faecal screening of other bird species, both native and exotic, which have contact with the hihi at the N.W.C., would be very valuable.

Reference List

Adie, J. R. (1908). Note on a parasite in the sparrow . Ind. Med. Gazette, 43, 176-180.
as cited by Wolfson, F. (1940) Organisms described as avian *Toxoplasma,* Am. J. Hyg.32 p. 88-99.

Aragão, H. d. B. (1911). Beobachtungen über Hämogregarinen von Vögeln. Mem. D. Inst. Osw. Cruz. 3, 54-64.
as cited by Wolfson, F. (1940) Organisms described as avian *Toxoplasma,* Am. J. Hyg.32 p. 88-99.

Aragão, H. d. B. (1933). Considerations sur les Hemogregarines des oiseaux. Compt. Rend. Soc. Biol. 113(214-215).
as cited by Wolfson, F. (1940) Organisms described as avian *Toxoplasma,* Am. J. Hyg.32 p. 88-99.

Baker, D. G., Speer, C. A., Yamaguchi, A., Griffey, S. M., & Dubey, J. P. (1996). An unusual coccidian parasite causing pneumonia in a Northern Cardinal (*Cardinalis cardinalis*). J. Wildl. Dis., 32(1), 130-132.

Baker, J. R., Bennett, G. F., Clark, G. W., & Laird, M. (1972). Avian Blood Coccidians. Adv. Parasitol., 10, 1-30.

Baker, J. R., Lainson, R., & Killick-Kendrick, R. (1959). *Lankesterella corvi* n. sp., a blood parasite of the English rook, *Corvus f. frugilegus* L.. J. Protozool., 6, 233-238.
as cited by Levine, N.D. (1982) The Genus *Atoxoplasma* (Protozoa, Apicomplexa) J. Parasitol., 68(4), 719-723 .

Ball, S. J., Brown, M. A., Daszak, P., & Pittilo, R. M. (1998). *Atoxoplasma* (Apicomplexa: Eimeriorina: Atoxoplasmatidae) in the greenfinch (*Carduelis chloris).* J. Parasitol., 84(4), 813-817.

Barré, N., & Troncy, P. M. (1974). Note on a coccidia of some Ploceidae in Chad: *Isospora xerophila* n. sp. Z. Parasitenk., 44, 139-147.
as cited by Svobodová (1994) *Isospora, Caryospora* and *Eimeria* (Apicomplexa: Eimeriidae) in passeriform birds from Czech Republic, Acta Protozoologica 33 p. 101-108.

Batyrshina, N. K., & Kazykhanova, B. M. (1976). Infection of Passeriformes with coccidia of the genus *Isospora.* Materialy II Vsesoyuznogo S"ezda Protozoologov. Chast' 1. Obshchaya protozoologiya. Kiev, USSR: "Naukova Dumka".

Blanc, E., & Grulet, O. (1985). *Isospora vagoi* n. sp. parasitic in *Poephila guttata.* Bulletin Du Museum National D'Histoire Naturelle, A, 7(2), 401-405.

Boughton, D. C. (1937). Studies on oocyst production in avian coccidiosis. I. A dilution count technique. Am. J. Hyg., 25(2), 187-202.

Boughton, D. C. (1937). Studies on oocyst production in avian coccidiosis. II. Chronic Isosporan infections in the sparrow. Am. J. Hyg., 25(2), 203-211.

Box, E. D. (1966). Blood and tissue protozoa of the English Sparrow (*Passer domesticus domesticus*) in Galveston, Texas . J. Protozool., 13(2), 204-208.

Box, E. D. (1967). Influence of *Isospora* infections on patency of avian *Lankesterella* (Atoxoplasma, Garnham, 1950). J. of Parasitol., 53(6), 1140-1147.

Box, E. D. (1970). *Atoxoplasma* associated with an Isosporan oocyst in canaries. J. Protozool., 17 , 391-396.

Box, E. D. (1975). Exogenous stages of *Isospora serini (Aragao)* and *Isospora canaria* sp. n. in the canary (*Serinus canarius* Linnaeus). J. of Protozool., 22(2), 165-169.

Box, E. D. (1977). Life cycles of two *Isospora* species in the canary (*Serinus canarius)* Linnaeus. J. Protozool., 24(1), 57-67.

Castro, I. (1995). Behavioural Ecology and Management of hihi (*Notiomystis cincta)* an endemic New Zealand honeyeater. Unpublished doctoral dissertation, Massey University, Palmerston North.

Castro, I. (2000). Nothing in life is that easy! An historical review of hihi (stitchbird) conservation and research in New Zealand . Proceedings of the joint conference of the Wildlife Disease Association (Australasian Section) and Wildlife Society of the New Zealand Veterinary Association .

Cheissin, E. M. (1947). Variability of the oocysts of *Eimeria magna* Pérard. Zool.Zh., 26, 17-30.

Cheissin, E. M. (1957). Variability of the oocysts of *Eimeria intestinalis* Heissin, 1948, a parasite of domestic rabbit. Vest. Leningr.Univ., Ser. Biol., 9, 43-52.
(In Russian) as cited by Svobodová (1994) *Isospora, Caryospora* and *Eimeria* (Apicomplexa: Eimeriidae) in passeriform birds from Czech Republic. Acta Protozoologica 33: 101-108.

Coleman, J. D. (1974). The use of artificial nest sites erected for starlings in Canterbury, New Zealand . N.Z. J. Zool., 1 (3), 349-54.

Cooper, J. E., Gschmeissner, S., & Greenwood, A. G. (1989). Atoxoplasma in greenfinches (*Carduelis chloris*) as a possible cause of "going light". Vet. Record, 124, 343-344.

Cork, S. C., Alley, M. R., & Stockdale, P. H. G. (1995). A quantitative assessment of haemosiderosis in wild and captive birds using image analysis. Avian Path., 24(2), 239-254.

Corradetti, A., & Scanga, M. (1963). *Atoxoplasma coccothraustis* n. sp., parassita dei frosone (*Coccothraustes coccothraustes*) . Parasitologia, 5, 61-72.
as cited by Levine, N.D. (1982) The Genus *Atoxoplasma* (Protozoa, Apicomplexa) J. Parasitol., 68(4), 719-723 .

Correa, C. (1928). Contribuicao ao estudo das hemogregarinas do Brazil. Rev. Biol. Hyg. S. Paulo, 1, 75-77.
as cited by Levine, N.D. (1982) The Genus *Atoxoplasma* (Protozoa, Apicomplexa) J. Parasitol., 68(4), 719-723 .

Current, W. L., Upton, S. J., & Long, P. L. (1990). Coccidiosis of man and domestic animals . Boca Raton, Florida, U.S.A.: CRC Press, Inc.

De Mello, I. F. On haemoprotozoa of Indian birds. C.R. 12th Internat. Congr. Zool.
as cited by Levine, N.D. (1982) The Genus *Atoxoplasma* (Protozoa, Apicomplexa) J. Parasitol., 68(4), 719-723 .

Desser, S. S. (1980). An ultrastructural study of the asexual development of a presumed *Isospora* sp. in mononuclear, phagocytic cells of the evening grosbeak (*Hesperiphona vespertina*) . J. Parasit., 66(4), 601-612.

Dissanaike, A. S. (1967). *Lankesterella lainsoni* sp. nov. from the Ceylon Mynah bird (*Acridotheres tristis melanosternus)*. Ceylon J. Sci. Biol. Sci., 6 , 225-229.

Doran, D. J. (1978). The life cycle of *Eimeria dispersa* Tyzzer 1929 from the turkey in gallinaceous birds. J. Parasit., 64(5), 882-885.

Doré, A. B. (1918). Rat trypanosomes in New Zealand. N.Z. Jour. Sci. Tech., 1(4), 200.
as cited by Laird, M. (1950). Some blood parasites of New Zealand birds. Zoology Publications, Victoria University College, No. 5.

Dubey, J. P. (1975). Experimental *Isospora canis* and *Isospora felis* infection in mice, cats, and dogs. J. Protozool., 22, 416-417.

Dubey, J. P. (1978). Life cycle of *Isospora ohioensis* in dogs. Parasitology, 77, 1-11.

Dubey, J. P. (1979). Life cycle of *Isospora rivolta* (Grassi 1879) in cats and mice. J. Protozool., 26, 433-443.

Dubey, J. P., & Frenkel, J. K. (1972). Extra-intestinal stages of *Isospora felis* and *I. rivolta* (Protozoa:Eimeriidae) in cats. J. Protozool., 19(1), 89-92.

Fayer, R. (1980). Epidemiology of protozoan infections: the coccidia. Vet. Parasit., 6, 75-103.

Flammer, K., Butterworth, S. A., & Whitt, D. A. (1989). Atoxoplasmosis in canaries. AFA Watchbird, August/September, 24-26.
as cited by Norton *et al* (1995) Bali Mynah captive medical management and reintroduction program, Assoc. Avian Vet., Main Conf. Proc., 1995, p125-136.

Gardner, S. L., & Duszynski, D. W. (1990). Polymorphism of eimerian oocysts can be a problem in naturally infected host: an example from subterranean rodents in Bolivia. J. Parasitol., 76, 805-811.
as cited by Svobodová (1994) *Isospora, Caryospora* and *Eimeria* (Apicomplexa: Eimeriidae) in passeriform birds from Czech Republic. Acta Protozoologica 33: 101-108.

Garnham, P. C. C. (1950). Blood parasites of East African vertebrates, with a brief description of exo-erythrocytic schizogony in *Plasmodium pitmani*. Parasitology, 40, 328-337.

Greiner E.C., Bennett, G. F., White, E. M., & Coombs, R. F. (1975). Can. J. Zool., 53, 1762-1787.

Grulet, O., Landau, I., Millet, P., & Baccam, D. (1986). Les *Isospora* du Moineau, II - Études sur la biologie. Ann. Parasitol. Hum. Comp., 61(2), 161-192.

Harvey, C. J., Rideout, B. A., Papendick, R. E., Sutherland-Smith, M. R., Stalis, I. H., & Gardiner, C. H. (1997). Atoxoplasmosis in Indochinese white-rumped shamas (*Copsychus malabaricus interpositus*). Abstracts of the 40th Annual Meeting of the American Society of Veterinary Laboratory Diagnosticians, Louisville, Kentucky, U.S.A., October 1997, 21.

Helman, R. G., Jensen, J. M., & Russell, R. G. (1984). Systemic protozoal disease in zebra finches. J. Am. Vet. Med. Assoc., 185, 1400-1401.

Hendrix, C. M. (1998). Diagnostic Veterinary Parasitology, 2nd Ed. St Louis, U.S.A.: Mosby Inc.

Joyner, L. P., & Davies, S. F. M. (1960). Detection and assessment of sublethal infections of *Eimeria tenella* and *Eimeria necatrix* . Exptl Parasitol., 9, 243-249.

Khan, R. A., & Desser, S. S. (1971). Avian *Lankesterella* infections in Algonquin Park, Ontario. Can. J. Zool., 49, 1105-1110.

Lainson, R. (1959). *Atoxoplasma* Garnham, 1950, as a synonym for *Lankesterella* Labbe, 1899. Its life cycle in the English sparrow (*Passer domesticus domesticus*, Linn.). J. Protozool., 6(4), 360-371.

Laird, M. (1950). Some blood parasites of New Zealand birds . Zoology Publications, Victoria University College, 5, 1-20.

Laird, M. (1959). *Atoxoplasma paddae* (Aragao) from several south pacific silvereyes (*Zosteropidae*) and a New Zealand rail. J. Parasit., 45, 47-52.

Laveran, A. (1900). Au sujet de l'hematozoaires endoglobulaire de *Padda orizivora*. C. R. Soc. Biol., 52, 19.
as cited by Box, E.D.(1967) Influence of *Isospora* infections on patency of avian *Lankesterella* (*Atoxoplasma*, Garnham, 1950), J. Parasit. 53(6) p. 1140-1147.

Levine, N. D. (1974). Historical aspects of research on coccidiosis. Proceedings of the Symposium on Coccidia and Related Organisms, Guelph, Ontario , 1-10.

Levine, N. D. (1982). The genus *Atoxoplasma* (Protozoa, Apicomplexa). J. Parasitol., 68(4), 719-723.

Levine, N. D. (1988). The Protozoan Phylum Apicomplexa, Vol. 1 . Boca Raton, Florida: CRC Press Inc.

Lillehoj, H. S., & Trout, J. M. (1993). Coccidia: a review of recent advances on immunity and vaccine development. Avian Pathology, 22, 3-31.

Lindsay, D. S., & Blagburn, B. L. (1994). Biology of Mammalian *Isospora*. Parasitology Today, 10(6), 214-219.

Lindsay, D. S., Dubey, J. P., Toivio-Kinnucan, M. A., Michiels, J. F., & Blagburn, B. L. (1997). Examination of extraintestinal tissue cysts of *Isospora belli* . J. Parasit., 83(4), 620-625.

Long, P. L., & Joyner, L. P. (1984). Problems in the identification of species of *Eimeria*. J. Protozool., 31, 537-541.

Mackerras, M. J., & Mackerras, I. M. (1960). The haematozoa of Australian birds. Austral. J. Zool., 8, 226-260.
as cited by Levine, N.D. (1982) The Genus *Atoxoplasma* (Protozoa, Apicomplexa) J. Parasitol., 68(4), 719-723 .

Martinez, F., & Muñoz, E. (1998). *Atoxoplasma* spp. in a hybrid passerine (*Serinus canarius* X *Carduelis cannabina*) . Avian Pathology, 27, 420-422.

Marullez, M. (1913). Au sujet d'un *Toxoplasme* dis oiseaux. Bull. Soc. Path. Exot., 6, 323-326.
as cited by Wolfson, F. (1940) Organisms described as avian *Toxoplasma,* Am. J. Hyg.32 p. 88-99.

Mazgajski, T. D., & Kedra, A. H. (1998). Endoparasite *Isospora* sp. (Coccidia, Eimeriidae) affects the growth of starling (*Sturnus vulgaris)* nestling. Acta Parasitologica, 43(4), 214-216.

McNamee, P., Pennycott, T., & McConnell, S. (1995). Clinical and pathological changes associated with atoxoplasma in a captive bullfinch (*Pyrrhula pyrrhula*). Vet. Record, 136, 221-222.

Mesher, C. I., & Mauldin, E. A. (1996). What is your Diagnosis? Five Juvenile Canaries. Vet. Clin. Path., 25(4), 140 & 151.

Michiels, J. F., Hofman, P., Bernard, E., St. Paul, M. C., Boissy, C., Mondain, V., LeFichoux, Y., & Loubiere, R. (1994). Intestinal and extraintestinal *Isospora belli* infection in an AIDS patient. Path. Res. Prac., 190, 1089-1093.
as cited by Lindsay, D.S.; Dubey, J.P.; Toivio-Kinnucan, M.A.; Michiels, J.F.; Blagburn, B.L. (1997) Examination of extraintestinal tissue cysts of *Isospora belli*, J. Parasitol. 83(4) p. 620-625
.

Milde, K. (1979). Light and Electron Microscopic Studies on Isosporan Parasited (Sporozoa) in Sparrows (*Passer Domesticus* L.). Protistologica, 15, 607-627.

Myers, J. G. (1923). The present position of the endemic birds of New Zealand. N.Z. Jour. Sci. Tech., 6(2), 65-99.
as cited by Laird, M. (1950). Some blood parasites of New Zealand birds. Zoology Publications, Victoria University College, No. 5.

Norton, T. M., Seibels, R. E., Greiner, E. C., & Latimer, K. S. (1995). Bali Mynah Captive Medical Management and Reintroduction Program. Assoc. Avian Vet., Main Conf. Proc., 125-136.

Oliver, W. R. B. (1930). New Zealand birds. Wellington: Fine Arts (N.Z.), Ltd, Wellington .
as cited by Laird, M. (1950) Some blood parasites of New Zealand birds. Zool. Publ. Vict. Univ. Coll., Wellington No. 5: 1-20.

Panigahy, B., & Senne, D. A. (1991). Diseases of mynahs: review article. J. Am. Vet. Med. Assoc., 199(3), 378-381.

Partington, C. J., Gardiner, C. H., Fritz, D., Phillips, L. G., & Montali, R. J. (1989). Atoxoplasmosis in Bali Mynahs (*Leucopsar rothschildi*). J. Zoo Wildl. Med. 20(3), 328-335.

Poelma, F. G., Zwart, P., & Strik, W. J. (1971). Lankesterella (Atoxoplasma, Garnham, 1950) infections in birds in the Netherlands. Neth. J. Vet. Sci., 4(1), 43-50.

Poon, S. K., & Chew, W. K. (1991). *Isospora corvi* Ray, Shivnani, Oommen and Bhaskaran, 1952 from the common house crow (*Corvus splendens Vieillot*) of Selangor, Peninsular Malaysia . Folia Parasitologica, 38(3), 201-207.

Prattis, S. M., Cioffee, C. J., Reinhard, G., & Zaoutis, T. E. (1990). A retrospective study of disease and mortality in zebra finches. Lab. Anim. Sci., 40(4), 402-405.

Quiroga, M. I., Aleman, N., Vazquez, S., & Nieto, J. M. (2000). Diagnosis of atoxoplasmosis in a canary (*Serinus canarius*) by histopathologic and ultrastructural examination. Avian Diseases, 44, 465-469.

Restrepo, C., Macher, A. M., & Radany, E. H. (1987). Disseminated extraintestinal isosporiasis in a patient with acquired immune deficiency syndrome. American Journal of Clinical Pathology, 87, 536-542.
as cited by Lindsay, D.S.; Dubey, J.P.; Toivio-Kinnucan, M.A.; Michiels, J.F.; Blagburn, B.L. (1997) Examination of extraintestinal tissue cysts of *Isospora belli*, J. Parasitol. 83(4) p. 620-625

Rosenbusch, F. (1932). Toxoplasmosis avium en los canarios. Reunión Soc. Argent. Patol. Reg. Norte, 2, 904-906.

as cited by Laird, M. (1959) *Atoxoplasma paddae* (Aragão) from several south Pacific silvereyes (*Zosteropidae*) and a New Zealand rail. J. Parasitology 45: 47-52 .

Rossi, G., Perrucci, S., & Macchioni, G. (1996). *Isospora atrata* N.Sp. (Apicomplexa, Eimeriidae): a new coccidium isolated from *Carduelis atrata* (Passeriformes, Fringillidae). J. Euk. Microbiol., 43(6), 489-491.

Rossi, G., Perrucci, S., Taccini, E., Vitali, C. G., Braca, G., & Renzoni, G. (1997). Mortality in black siskins (*Carduelis atrata*) with systemic coccidiosis. J. Wildl. Dis., 33(1), 152-157.

Rousselot, R. (1953). Notes de parasitologie tropicale, Vol 1 . Parasites Du Sang Des Animaux (Systeme Reticuloendothelial), 152.
as cited by Levine, N.D. (1982) The Genus *Atoxoplasma* (Protozoa, Apicomplexa) J. Parasitol., 68(4), 719-723 .

Stuart, B. P., & Lindsay, D. S. (1986). Coccidiosis in swine. Vet. Clin. N. Am. Food Anim. Pract., 2, 455-468.

Svobodová, M. (1994). *Isospora, Caryospora* and *Eimeria* (Apicomplexa: Eimeriidae) in passeriform birds from Czech Republic. Acta Protozoologica, 33, 101-108.

van Riper III, C., van Riper, S., & Laird, M. (1987). Discovery of *Atoxoplasma* in Hawaii. J. Parasit., 73(5), 1071-1073.

Wagner, A., & Ruedi, D. (1981). Nachweis von Endoparasiten bei Vogeln des Zoologischen Gartens Basel. Welche Rolle spielen Wildvogel als Ubertrager. Schweizer Archiv Fur Tierheilkunde, 123(9), 467-481.

Wolfson, F. (1940). Organisms described as avian *Toxoplasma* . Am. J. Hyg., 32, 88-99.

Zasukhin, D. N., Vasina, S. G., & Levitanskaya, P. B. (1957). K voprosy ob atoksoplazmakh u ptits [On the problem of the atoxoplamas of birds.] . Trudy Leningr. Obshch. Estest., 73, 117-120.

APPENDIX I

Hihi management at the N.W.C.

The hihi at the N.W.C. are housed in open-air aviaries which have wire mesh on all sides including the roof, and a natural dirt/leaf litter floor. There is natural vegetation growing within and around the aviaries and this is kept at a level to provide a comfortable habitat and shelter for the birds while allowing sunlight to penetrate in order to reduce the proliferation of fungi. All the hihi aviaries are divisible into at least 2 flights, a capacity which allows birds held together in groups or pairs to be separated easily if conflict or stress is occurring and to be held adjacent to each other without direct contact . The birds are generally grouped singly or in pairs and can be held in aviaries which are not available to public viewing. Hihi feed on nectar, fruits and insects. In addition to the foods available naturally in their aviaries, they are supplemented daily with jam water (containing bee pollen and Pronutra cereal (National Brands LTD, Wadeville, South Africa)), a commercial honeyeater mix (Wombaroo, Wombaroo Food Products, Adelaide, Australia), and a fruit/vegetable puree. The birds' diets are also supplemented with invertebrates when available during the breeding and nestling periods. Roostboxes and artificial nestboxes are provided and these are cleaned and/or changed often to limit the build-up of faeces, foodstuffs, and, in the case of nestboxes, mites. The nestboxes are placed in areas which are easily accessible for cleaning and observing but at least risk from public disturbance. Inside nestboxes, the chicks are placed in artificial nests once the natural nest becomes soiled. All hihi are observed daily and any change in behaviour or signs of illness, are noted. Remote automatic bird weighers (Weighing Systems LTD, Nelson) are used to monitor bodyweights, a reduction in bodyweight being an important indicator of sickness.

APPENDIX II

Results of faecal examinations of N.W.C. hihi during the 1997-1998 season

Table 1: Results of faecal examinations of chicks/juveniles during Jan 1998

Treatment/Activity	Sample Date	No. of oocysts per sample[a]
date of hatch= 30-31/12/97	January 9	+++
P(1) 3 nestlings; T(5) dam; T(1) 1 nestling	11	++
	12	++
	13	++
	15	0
18th- P(1) nestlings	17&18	242
	19	220
	20	1650
T(5) nestlings; T(5) dam	21	4+
	22	2+
	23	+
	24	1 only
	25	0
	26	0
F 2 chicks	27	0
	28	0

[a] = 3 methods of recording were used in this month because 3 different people analysed samples: a) scale of +, ++, +++, ++++; b) scale of - 1+, 2+, 3+, 4+, 5+; c) actual numbers
P(1) = single primiquin dose; primiquin (NZ Defence Force) was used as a treatment for coccidiosis; the regime used at the N.W.C. was to treat twice per week until shedding was eliminated, at a dose rate of 1mg/kg orally
T(1) = toltrazuril (Baycox solution 2.5%, Bayer) treatment for a single day; toltrazuril was used as a treatment for coccidiosis; the dose rate used at the N.W.C. was 20mg/kg
T(5) =toltrazuril treatment for 5 days
F = fledging

Additional treatments: primiquin: nestlings 14 Jan, single dose

Table 2: Results of faecal examinations of chicks/juveniles during Feb-March 1998 before separation from dam

Treatment/Activity	Sample Date		No. of oocysts per sample/no. of *Capillaria* eggs per sample[a]
	February	8&9	0/5
		11	0/0
		12	0/1
		16	0/3
		17	0/20
		18	0/11
I(1) dam; I(1) fledglings		19	0/48
		20	0/23
		21	0/27
		22	0/28
		23	0/21
		24	0/11
		25	4/12
I(5) dam; I(5) fledglings		26	23/12
		27	101/9
		28	58/4
T(5) fledglings; T(5) dam	March	1	159/20
		2	54/38
		3	0/6
		4	0/60
		5	0/4
		6	0/10
		7	0/0
		8	0/14
		9	0/12
		10	0/30
		11	0/20
		12	0/22
		13	0/21
		14	0/14
		15	0/20
		16	0/9
		17	0/9
		18	0/34
		19	0/24
		22	0/46

[a] = all samples are pooled with samples from the dam "Jill"
I(1)= ivermectin (Ivomec, MSD-AgVet) treatment for a single day; ivermectin was used as a treatment for *Capillaria* and the dose rate used at the N.W.C. was 2mg/kg
I(5)= ivermectin treatment for 5 days
T(5)= toltrazuril treatment for 5 days

Additional treatments: toltrazuril: dam & fledgings 10-14 Feb

Table 3: Results of faecal examinations of chick/juveniles in 1998 after separation from dam

All results are shown as number of oocysts per sample/number of *Capillaria* eggs per sample

Sample Date	"Smith"	"Sprite"	"Tracy"	"Rachel"
April 2		0/13	0/1	0/0
14	0/0	0/17		0/9
22	0/0	0/10		0/0
29		0/4		0/3
May 12		0/0		
15		0/0		
June 5				0/0
July 10				0/0
21				0/0
Aug 4				0/0
17				0/0
Sept 11				0/0 n
29				0/0

n <0.02g faeces

Additional note: "Tracy" died on 7 April 98 from thoracic and myocardial haemorrhage of unknown cause

Table 4: Results of faecal examinations of adults during Jan-March 1998

All results are shown as number of oocysts per sample/number of *Capillaria* eggs per sample

Treatment/Activity	Sample Date	"Jill"	"Ray"	"GB"
	Jan 12		0/0	0/0
	13	+ [a]		
	19	0/1		
	20		0/0	0/0
	28	0/1		
	29	0/1		
	30	0/6		
	31	0/0		
	Feb 1	0/0		
	2	0/3		
	4	0/0		
	5	0/0		
	6	0/0		
	7	0/0		
	8	0/0		
	9	0/2		
T(5) Jill	10	0/1		
	11	0/0		
	15	0/0		
	17	0/20		
	18[b]	0/11		
I(1) Jill	19	0/48		
	20	0/23		
	21	0/27		
	22	0/28		
	23	0/21		
	24	0/11		
	25	4/12		
I(5) Jill	26	23/12		
	27	101/9		
	28	58/4		
T(5) Jill	March 1	159/20		
	2	54/38		
	3	0/6		
	4	0/60		
	5	0/4		
	7	0/0		
	8	0/14		
	9	0/12		
	10	0/30		
	11	0/20		
	12	0/22		
	13	0/21		
	14	0/14		
	15	0/20		
	16	0/9		
	17	0/9		
	18	0/34		
	19	0/24		
	22	0/46		

[a] = recording system used on this occasion involved the scale: +, ++, +++, ++++
[b] = from February 18th until the end of March, the samples from "Jill" were all pooled with those from the chicks
T(5)= toltrazuril treatment for 5 days
I(1)= ivermectin treatment for a single day
I(5) ivermectin treatment for 5 days

Additional treatments: toltrazuril: " Jill" 11-15 Jan, 21-25 Jan

Table 5: Drug treatments of "Jill" and her chicks during 1997-1998 season

	Toltrazuril	Ivermectin	Primiquin
Jill	11-15 Jan 98 21-25 Jan 98 10-14 Feb 98 1-5 March 98	19 Feb 98 26 Feb-2 March 98	
Chicks	11 Jan 98- 1 chick 21-25 Jan 98 10-14 Feb 98 1-5 March 98	19 Feb 98 26 Feb-2 March 98	11 Jan 98- 3 chicks 14 Jan 98- 4 chicks 18 Jan 98- 4 chicks

Table 6: Results of faecal examinations of adults during July-August 1998

All results are shown as number of oocysts per sample/number of *Capillaria* eggs per sample

Sample Date	"Ray"	"Jill"	"Keith"	"Ngatiwai"	"Rachel"
July 10	0/0	0/0	0/0	no samples taken	0/0
20/21	0/0	0/0	65/0		0/0
22			34/0		
24			4/0		
25			3/0		
26			#		
30			#		
Aug 4	0/0	0/0	132/0		0/0
17	0/0	0/0	59/0		0/0
21			66/0		

= see Appendix III, Table 1

Notes:
Treatments: itraconazole: Keith 9/7-24/7, 30/7-5/8
Activities: Keith moved to the N.W.C. 31/7
Further sampling: from Jill, Ray & Rachel pooled - 5 June - 0/0
16 June - 1/0

Table 7: Results of faecal examinations of adults during August-December 1998

All results are shown as number of oocysts per gram/number of *Capillaria* per gram (weight of faeces examined)

Sample Date	"Ray"	"Jill"	"Keith"	"Ngatiwai"	"Rachel"	"Hauturu"
Aug 28			10/0 (1.51)[a]	5/7 (w)		5/7 (w)p
Sept 11	0/0 (0.16)	0/0 (0.42)	0/0 (0.33)	0/3 (0.32)	0/0 (n)	
29	2/0 (0.56)	0/0 (0.15)	120/0 (0.3)	1280/0 (0.82)	0/0 (0.2)	19/0 (n)
Oct 2				2240/0 (1.04)	227/0 (0.11)	0/0 (0.1)
5	0/0 (0.42)	0/0 (0.53)	8/0 (0.49)	223/0 (0.17)	0/0 (0.34)	
7	0/0 (0.1)	0/0 (0.13)	0/0 (0.13)	0/0 (0.05)	0/0 (0.04)	0/0 (0.05)
13	0/0 (0.48)	0/0 (0.2)	0/0 (0.28)	953/16 (0.55)	0/0 (0.65)	
16				3370/60 (0.1)		
19				3/0 (0.32)		
20		0/0 (0.17)	0/0 (0.25)		0/8 (0.13)	
27	0/0 (0.12)	0/0 (0.24)	0/0 (n)	0/0 (0.18)	0/0 (0.21)	
30-2/11	0/0 (0.71)	0/0 (0.65)	42308/0 (0.52)[b]	0/5 (0.39)	0/0 (1.87)	
Nov4-6	0/0 (0.42)	0/0 (0.29)	78571/0 (0.14)[b]	0/0 (2.07)	0/0 (0.5)	
9	0/0 (0.4)	0/0 (0.5)	39286/0 (0.14)[b]	0/0 (1.77)		
10-11	0/0 (0.29)	0/0 (0.95)	[a]	0/4 (1.35)		
12	0/0 (0.11)	0/0 (0.3)				
14-16	0/0 (0.66)	0/0 (1.25)			0/6 (0.32)	
17-18	0/0 (0.35)		0/0 (0.24)		0/0 (0.17)	
24	0/0 (w)	0/0 (w)	0/0 (w)	0/0 (w)	0/0 (w)	
27	0/0 (w)		0/0 (w)	0/0 (w)	0/0 (w)	
Dec 4	0/0 (w)			0/0 (w)	0/0 (w)	
7			17770/0 (0.26)[b]			
8	0/0 (0.04)		49310/0 (0.29)[b]	0/0 (0.2)	0/0 (0.29)	
9			5833/0 (0.24)[b]			
10					0/0 (0.17)	
11			167/13 (0.3)[b]			

w = faecal weight not recorded
[a] = see Appendix III, Table 2
n <0.02g faeces
[b] = indicates uncertainty over whether structures are oocysts
p = samples from Ngatiwai & Hauturu pooled
Note: For a full list of treatments for this period, refer to Appendix II, Table 4

APPENDIX III

Results of faecal examinations of N.W.C. hihi during the 1998-1999 season

Table 1: Results of faecal examinations of chicks/juveniles during Dec 1998 and Jan 1999 before separation of the first clutch

Treatment/Activity	Sample Date	Oocysts per gram/*Capillaria* eggs per gram (weight of faeces examined)
date of hatch= 1/12/98	Dec 5&6	0/0 (0.32)
	7	0/0 (0.46)
	8	0/0 (0.27)
	9	0/0 (0.69)
	10	0/0 (1.35)
	11	0/0 (0.72)
	12	0/0 (0.66)
	13&14	0/0 (0.70)
	15	0/0 (0.70)
	16	0/0 (0.52)
	17	0/0 (1.05)
	18	0/0 (1)
	19	0/0 (1.37)
	20	0/0 (0.59)
watery faeces noted	21	0/0 (1.51)
banding of chicks	22	0/0 (0.88)
	23	10/0 (w)
T(5)	24	4/0 (0.48)
	25	2/0 (0.87)
	26	0/0 (1.34)
	27	0/0 (0.84)
chicks fledged	28	0/0 (0.54)
	29	0/0 (0.15)
	30	0/0 (0.08)
	31	0/0 (n) p
	Jan 1	0/0 (0.05) p
	2	0/0 (0.2)
	4	0/0 (n)
	8	0/0 (0.06)
	9	0/0 (0.08) p
	10	0/1 (0.02) p
	11	0/0 (0.04)p
	12	0/0 (0.04)
	14	0/0 (0.08)
	15	0/0 (0.31)
	16	133/0 (0.06)
	18	33/0 (0.24)
	19	14/0 (0.70)

T(5) = toltrazuril treatment for 5 days; w = weight not recorded; n <0.02g faeces; p = sample pooled with dam's

Table 2: Results of faecal examinations of chicks/juveniles in 1999 after separation of the 1st clutch

All results are shown as number of oocysts per gram/ number of *Capillaria* eggs per gram (weight of faeces examined)

Treatment /Activity	Sample Date	"Rangi"	"Roz"	"Missy"	"Watson"	"Sally"	small chick
T(5)	Jan 20	0/0 (0.03)		0/0 (0.27)	0/0 (0.02)		
	21	0/0 (0.12)		0/0 (0.3)	0/0 (0.03)		
	22	0/0 (n)	0/0 (0.04)	0/0 (n)	0/0 (n)		
	24	0/0 (0.14)	0/0 (n)	0/0 (n)	0/0 (n)		
	26	0/0 (0.77)		0/0 (0.15)	0/0 (0.22)		
	28	0/0 (n)	0/0 (0.1)	0/0 (0.08)			
	29			0/33 (0.06)			
	31	0/0 (n)	0/0 (n)	0/0 (n)	0/0 (n)		
	Feb 2	0/0 (0.05)		10923/0 (0.13)			
T(5)	3	0/0 (0.56)	0/0 (n)	28600/0 (0.09)	0/0 (n)		
	4	0/0 (0.15)	0/0 (n)	1282/9 (0.22)	0/0 (0.14)		
	5	19/0 (0.21)	0/0 (n)	400/0 (0.06)	0/0 (n)		
	6		0/0 (0.07)		0/0 (0.02)		
	7	0/0 (0.02)		0/0 (0.09)			
	8	0/0 (n)	0/0 (n)	13/0 (0.31)	0/0 (0.27)		
	9	0/0 (0.13)	0/0 (0.06)	0/0 (0.22)	0/0 (0.2)		
	10	0/0 (0.2)	0/0 (0.06)	364/5 (0.44)	0/0 (0.5)		
	11			85922/122 (0.09)			
	12	0/0 (0.04)		8360/0 (0.05)	0/0 (0.05)		
	14	0/0 (0.02)		58575/0 (0.04)	0/0 (0.04)		
T(5) Missy	15	1216/0 (0.38)	0/0 (0.08)	19486/19 (0.21)	0/0 (0.03)		
	16	0/0 (0.05)	0/2 (n)		0/0 (0.03)		
T(2)Rangi 2nd clutch hatches 17th&18th	17	181/0 (0.21)		0/0 (0.05)	0/0 (n)		
TTM	18		0/0 (0.03)	10/2 (n)			
	19		0/0 (n)	0/0 (n)			
	21		0/0 (n)	67/33 (0.18)			
	22		0/0 (n)	0/0 (n)			
	23					0/0 (0.19)p	
	24		0/0 (0.08)	10115/0 (0.13)		0/0 (0.05)p	

Treatment /Activity	Sample Date	"Rangi"	"Roz"	"Missy"	"Watson"	"Sally"	small chick
	Feb 25		0/0 (n)			0/0 (0.52)p	
T(5) Missy	26			0/0 (n)		0/0 (0.45)p	
	28		0/0 (n)	0/0 (n)		0/0 (1.64)p	
	March1					0/0 (n)p	
	2			0/0 (n)		0/0 (0.91)p	
	3		0/0 (0.12)	0/0 (n)		25/0 (0.71)	0/0 (n)
	4			0/0 (0.04)		107/0 (1.42)	0/0 (0.11)
T(5) 2nd clutch	5		0/0 (0.04)	0/0 (n)		87/0 (0.94)p	
	6		0/0 (n)	2/0 (n)		33/0 (1.2)p	
	7					0/0 (0.45)p	
S	8		0/15(0.26)	42/6(n)		5/0 (0.78)p	
	9		0/0 (n)	0/0 (0.14)		0/0 (1)	0/0 (0.79)
T(2) Missy D	10		0/0 (0.08)	38/0 (0.32)		0/0 (1.17)	0/0 (1.79)
	11		0/0 (0.04)	0/0 (0.19)		0/0 (1.45)	
	12		0/0 (n)	23/0 (0.26)		0/0 (0.98)	
	13			52/7(0.27)		0/0 (0.65)	
	14			19/0 (0.32)		0/0 (1.54)	
	15		0/0 (0.08)	17/0 (0.12)		0/0 (0.92)	
F Sally	16		0/0 (0.06)	51/0 (0.35)		0/0 (0.73)	
T(5) Missy	17		0/0 (0.06)	0/0 (0.04)			
	18		0/0 (n)	0/0 (n)		2/0 (n)	
	19		0/0 (n)	0/0 (n)			
	20		0/0 (0.04)	0/0 (n)			
	21		0/0 (n)	0/0 (0.07)			
	22		0/0 (0.04)	0/0 (0.07)		3100/0 (0.12)	
T(5)2nd clutch	23		0/0 (0.29)	0/0 (n)		0/0 (0.03)	
	24		0/0 (0.02)	0/0 (0.15)		289/0 (0.09)	
	25			0/0 (0.11)		20/0 (0.3)	
	26		0/0 (0.15)	0/0 (0.28)		0/0 (0.06)	
T(5) Missy	27		0/0 (0.06)	0/0 (0.42)		0/0 (0.07)	
	28			0/0 (0.22)		0/0 (0.03)	
	29		0/24(0.17)				
	30		0/0 (0.81)	0/0 (0.32)			
	31		0/0 (0.49)	0/0 (0.04)		0/67(0.03)	

Treatment /Activity	Sample Date		"Rangi"	"Roz"	"Missy"	"Watson"	"Sally"	small chick
	April	1		0/0 (0.5)	0/0 (0.18)		0/0 (0.06)	
		2			0/0 (0.06)		0/0 (0.02)	
		3					0/0 (n)	
		4					40/0 (0.05)	
		5					300/0 (n)	
		6		0/0 (0.61)				
T(5) Sally		9			0/0 (0.12)		933/0 (0.03)	
		13		0/5(0.74)	0/5(0.38)		0/0 (0.04)	
		14					0/0 (0.08)	
		15			0/0 (0.1)		0/0 (0.06)	
		18					0/0 (n)	
		20		636/0 (0.33)	0/0 (0.07)		0/20 (0.10)	
T(5) Roz		21		73/0 (0.71)				
		22		117/0 (0.65)				
		23		6/0 (0.70)	0/0 (0.27)			
		24		0/0 (0.95)			0/0 (0.11)	
		25		0/0 (1.34)			382/0 (0.22)	
		26		0/0 (0.93)			0/80 (0.05)	
		27		0/0 (0.62)	0/0 (0.2)		0/108(0.5)	
I(2) Sally		28					0/4(0.49)	
		29					0/0 (n)	
		30		0/0 (0.17)	0/0 (0.09)		0/0 (0.1)	
	May	3					0/38(1.38)	
		4		0/0 (0.75)	0/11(0.19)		0/32(1)	
		5					0/0 (0.99)	
I(2) Sally		6					0/0 (0.47)	
		7		71/0 (1.41)			0/17(0.36)	
T(5) Roz		8		2070/0 (0.74)[a]			0/200 (0.2)	
		9					0/225 (0.04)	
		10					0/32(1.18)	
		11		15/0 (0.53)	0/0 (0.63)		0/4(0.55)	
I(2) Sally		13		0/0 (1.19)			0/17(1.68)[b]	
		14		0/0 (1.27)[c]	0/0 (0.54)			
		15					0/6(1.58) ↔	
		16		0/0 (0.95)∝				
		18			0/0 (0.36)		0/54(1)Σ	

Treatment /Activity	Sample Date	"Rangi"	"Roz"	"Missy"	"Watson"	"Sally"	small chick
	May 20					0/122 (0.67)	
	21		0/0 (0.24)	0/2(0.87)		0/119 (0.54)	
	22					0/60 (1)	
	23					0/21(0.66)	
	25		0/0 (0.27)	0/0 (0.21)		0/0 (0.11)	
	28		0/0 (0.06)	0/0 (0.18)		0/6(0.34)	
	June 1		0/0 (0.43)	0/0 (0.09)		0/92(0.26)	
	4		0/0 (0.49)	0/0 (0.06)		0/6(0.33)	
	July 28		0/0 (0.03)	0/0 (0.26)		0/0 (0.2)	
	Sept 28		0/0 (0.4)	0/0 (0.1)		267/0 (0.51)	
	Oct 1					0/0 (0.52)	
	2&3					0/0 (0.18)	
	4					0/0 (0.23)	
	14					0/0 (0.21)	
	28			0/0 (0.3)		0/0 (0.02)	
	Nov 11			0/0 (0.03)		0/0 (0.12)	
	22			0/8(0.12)		0/0 (0.01)	
	26		0/0 (0.02)				
	30			0/0 (0.17)			
	Dec 1		0/0 (n)			0/0 (n)	
	8		0/0 (w)	0/0 (w)		0/0 (w)	

n <0.02g faeces (in these cases the number of oocysts &*Capillaria* eggs recorded are per sample); T(5) = toltrazuril treatment for 5 days; TTM = Rangi & Watson transferred to Tiritiri Matangi Island; S=separate smaller chick & begin hand-rearing it; D=smaller chick died; F=fledging; I(2) = ivomectin treatment for 2 days; p=samples from Sally & small chick pooled; w = faecal weight not recorded; [a] = samples from 8th, 9th &10th pooled, [b] = samples from 12th, 13th & 14th pooled; [c] = samples from 14th & 15th pooled; ↔ = samples from 15th & 16th pooled; ∝ = samples from 16th & 18th pooled; Σ = samples from 18th & 19th pooled.

Additional treatments: ivermectin: Sally 2-6 June

Table 3: Results of faecal examinations of adults in late 1998 and during 1999

All results are shown as number of oocysts per gram/number of *Capillaria* eggs per gram (weight of faeces examined)

Treatment/ Activity	Sample Date	"Ray"	"Jill"	"Keith"	"Ngatiwai"	"Rachel"
	Dec 7			17770/0 (0.26)[a]		
T(5) Keith	8	0/0 (0.04)		49310/0 (0.29)[a]	0/0 (0.2)	0/0 (0.29)
	9			5833/0 (0.24)[a]		
	10					0/0 (0.17)
	11			167/13 (0.3)[a]		
	12			114/29 (0.14)[a]		
	13			543/86 (0.14)[a]		0/50 (0.12)
	14			0/4 (0.23)		
	15			3929/18 (0.28)[a]		
	16	0/0 (0.81)		10/0 (0.21)[a]	0/32 (0.37)	0/0 (0.19)
	17			0/0 (0.18)		
I(1) Ngatiwai	18			0/38 (0.26)		
	21				0/49 (0.37)	
J Rachel & Keith	22	0/0 (0.11)		18500/50 (0.08)	0/8 (1.58)	0/16 (0.25)
	24			364/0 (0.11)		
	25			33/0 (0.06)		
	26			0/0 (0.41)		
	27			0/10 (0.21)		
	29	0/0 (0.07)	0/0 (n)	0/0 (0.05)	0/2 (0.96)	
	30		0/0 (0.06)			
	31		0/0 (n)p			
	Jan 1		0/0 (0.05)p			
	5	0/46 (0.13)	0/0 (0.09)	0/33 (0.12)	0/67 (0.15)	0/0 (0.04)
	9		0/0 (0.08)p			
	10		0/0 (0.03)			
	11		0/0 (0.04)p			
	12		0/0 (0.17)			
	13	0/6 (0.18)	0/0 (0.12)	0/0 (0.41)	0/5 (0.11)	0/0 (0.17)
	20	0/0 (0.09)	0/0 (0.12)	622/89 (0.09)		0/67 (0.06)
	26	0/11 (0.18)	0/0 (0.21)	0/0 (0.04)	0/10 (0.2)	0/59 (0.17)
J Jill & Ngatiwai	Feb 2	0/0 (1.24)	0/0 (0.33)	0/0 (0.67)	0/7 (0.29)	0/14 (0.29)
	9			0/7(0.29)	0/4 (0.52)	0/20 (0.1)
	16	0/0 (0.85)		0/0 (0.15)	0/0 (0.1)	0/20 (0.3)
	23	0/0 (0.44)				0/0 (032)
	March 2	0/0 (1.29)				0/0 (0.38)

Treatment/ Activity	Sample Date	"Ray"	"Jill"	"Keith"	"Ngatiwai"	"Rachel"
	March 9	0/0 (0.94)				0/0 (0.21)
	17	0/0 (0.9)	0/0 (0.59)		0/5 (0.37)	0/0 (0.58)
T(5) Jill	23	0/0 (1.61)			0/0 (0.16)	0/0 (1.34)
	24		0/0 (0.18)			
	31	0/0 (0.98)	0/0 (0.16)		0/0 (0.41)	0/0 (0.45)
	April 6	0/0 (0.58)	0/0 (0.36)		0/0 (0.72)	0/0 (0.31)
	13	0/0 (0.09)	0/0 (0.43)		0/0 (0.18)	0/0 (0.63)
	20	0/0 (0.44)	0/0 (0.4)		0/0 (0.4)	0/0 (0.66)
	27	0/0 (1.72)	0/0 (0.24)		0/0 (0.28)	0/0 (0.32)
I(2) Jill	28		0/0 (0.33)			
	May 4	0/0 (0.84)	0/0 (0.49)		0/0 (0.63)	0/0 (0.33)
	11	0/0 (1.81)	0/0 (0.28)		336/0 (0.28)	0/0 (0.58)
	13				9/0 (0.67	
	15				0/0 (0.12)	
	18	0/0 (0.51)	0/0 (0.14)		0/0 (0.6)	0/0 (0.76)
	21				0/0 (1.37)	
	25	0/0 (0.18)	0/130 (0.23)		0/0 (0.12)	0/0 (0.12)
	28		0/0 (0.19)		0/0 (0.45)	
	June 1	0/0 (1.15)	0/0 (0.45)		0/0 (0.8)	0/0 (0.15)
	July 28	0/0 (0.38)	0/0 (0.84)		0/0 (0.29)	0/0 (0.34)
	Sept 30		0/0 (0.52)		0/0 (0.15)	0/23 (013)
	Oct 18	0/0 (0.1)				
	28	0/0 (0.21)	0/0 (0.27)		0/0 (0.38)	0/0 (023)
	Nov 11	0/0 (0.06)	0/142 (0.12)		0/0 (0.63)	0/0 (0.24)
	22	0/0 (0.32)			0/19 (0.36)	0/11 (0.18)
	26		0/267 (0.03)			
	30	0/0 (0.21)	0/27 (0.44)		0/38 (0.13)	0/0 (0.35)
	Dec 8	0/0 (w)	0/3 (w)		0/0 (w)	0/0 (w)
	16	0/0 (0.45)	0/0 (0.2)		0/67 (0.18)	0/0 (0.08)
	29	0/0 (0.37)			0/0 (0.04)	0/0 (0.17)

T(5) = toltrazuril treatment for 5 days; J = join birds in same aviary; I(1) = ivermectin treatment for 1 day; I(2) = ivermectin treatment for 2 days; w = weight of faeces not recorded; n <0.02g faeces; p = samples pooled from Jill & chicks; [a] = indicates uncertainty over whether structures are oocysts

Additional treatments: toltrazuril: Keith 23-27 Dec 98, 22-26 Jan 99; Jill 9-13 April 99; ivermectin: Rachel 18&21 Jan 99; Jill 6&7 May 99; flucytosine[b]: Rachel 30 Nov 98-13 Dec 99, 16-25 Jan 99, 5-7 Feb 99

[b] = flucytosine (5-fluorocytosine, Alcobon 500mg tablets, Roche) is a treatment for aspergillosis and the dose rate used is 200mg/kg.

Table 4: Drug treatments of hihi during 1998-1999 season

Bird	Toltrazuril	Ivermectin	Flucytosine	Fluconazole[a]	Itraconazole
Keith	28 Aug-1 Sept 98 1-5 Oct 98 10-14 Nov 98 20-24 Nov 98 8-12 Dec 98 23-27 Dec 98 22-26 Jan 99		22-29 Sept 98		27 July-5 Aug 98
Ngatiwai	1-6 Sept 98 1-5 Oct 98 15-19 Oct 98 14-18 May 99	1-6 Sept 98 18 Dec 98 23-26 Sept 98	19-28 Sept 98	21-28 Sept 98	
Rachel		18&21 Jan 99	29 Aug-7 Sept 98 30 Nov-13 Dec 98 16- 25 Jan 99 5-7 Feb 99		
Jill	23-27 March 99 9-13 April 99	28&29 April 99 6&7 May 99 13&14 May 99 2-6 June 99			
Hauturu	1-6 Sept 98 2-6 Oct 98	1-6 Sept 98	21-30 Sept 98		
Moerangi[b] chicks- 1st clutch	1-3 Sept 98 24-28 Dec 98 20-24 Jan 99 3-7 Feb 99	1-3 Sept 98			
Missy	15-19 Feb 99 26 Feb-2 March 99 10&11 March 99 17-21 March 99				
Rangi chicks- 2nd clutch	17&18 Feb 99 5-9 March 99 23-27 March 99 9-13 April 99	28&29 April 99 6&7 May 99 13&14 May 99 2-6 June 99			
Roz	21-25 April 99 8-12 May 99				

[a] = fluconazole (Diflucan, Pfizer) is used as a treatment for aspergillosis
[b] = Moerangi was found dead on 4 Sept 1998

APPENDIX IV

Table 1: Results of faecal examinations of the N.W.C. hihi known as "Keith", up until August 21st 1998

Treatment/Activity	Sample Date	Oocysts per sample/*Capillaria* eggs per sample
Itraconazole[a] treatment approx. 9th & stopped 24th	July 10	0/0
	21	65/0
	22 4-5pm	34/0
	24 am	4/0
	25 entire day	3/0
	26 9am	0/0
	2pm	0/0
	5pm	2/0
Itraconazole started 27th	30 8-11am	0/0
	5pm	0/0
Moved to N.W.C.	31	
	Aug 4	132/0
Last dose itraconazole	5	
	17	59/0
	21	66/0

[a] = itraconazole (Sporanox, Janssen-Cilag Pty Ltd) is a human medication which was used as a prophylaxis and as a treatment for aspergillosis; the dosage regime used at the N.W.C. was 4 grains per bird per day which was crushed and sprinkled on top of jam water.

Table 2: Results of faecal examinations of the N.W.C. hihi known as "Keith", after August 21st 1998

Treatment/Activity	Sample Date	Oocysts per gram/*Capillaria* eggs per gram (weight of faeces examined)
	Aug 27 4.30pm	0/0 (n)
	27/28 night	10/0 (1.51)
T(5)	28 10am	0/0 (0.05)
	30	6/0 (0.32)
	31 am	0/0 (n)
	Sept 1	0/0 (0.01)
	2	0/0 (0.05)
	11	0/0 (0.33)
	29	120/0 (0.3)
T(5) 1st	2	227/0 (0.11)
	5	8/0 (0.49)
	7	0/0 (0.13)
	13	0/0 (0.28)
	27	0/0 (n)
	30-2/11	42308/0 (0.52)[a]
	Nov 4-6	78571/0 (0.14)[a]
	9	39286/0 (0.14)
T(5)	10 3.30pm	3850/0 (w)
	10/11 night	22000/0 (0.25)
	11 11.30am	1/0 (n)
	4pm	40/0 (n)
	11/12 night	2200/0 (0.05)
	12 11am	0/0 (n)
	13/14 night	36/0 (0.28)
	14/15 night	29/0 (0.21)
	15/16 night	18/0 (0.16)
	18	0/0 (0.24)
	24	0/0 (w)
	27	0/0 (w)
	Dec 7	17770/0 (0.26)[a]
T(5)	8	49310/0 (0.29)[a]
	9	5833/0 (0.24)[a]
	11	167/13 (0.3)[a]
	12	114/29 (0.14)[a]
	13	543/86 (0.14)[a]
	14	0/4 (0.23)
	15	3929/18 (0.28)[a]
	16	2/10 (0.21)[a]
	17	0/0 (0.18)
	18	0/38 (0.26)
	22	18500/50 (0.08)
T(5) 23rd	24	364/0 (0.11)
	25	33/0 (0.06)
	26	0/0 (0.41)
	27	0/10 (0.21)
	29	0/0 (0.05)
	Jan 5	0/33 (0.12)
	13	0/0 (0.41)
	20	622/89 (0.09)
	26	0/0 (0.04)
	Feb 2	0/0 (0.67)
	9	0/7 (0.29)
	16	0/0 (0.15)

n < 0.02g faeces (in these cases the number of oocysts & *capillaria* are per sample)
[a] = indicates uncertainty over whether structures are oocysts
w = weight of faeces not recorded
T(5) = toltrazuril treatment for 5 days

Additional treatments: flucytosine 22-29 Sept 98; itraconazole 20-24 Nov 98, 22-26 Jan 99

APPENDIX V

Pre-laying to post-hatching oocyst shedding by parents of chicks

1997-1998 season

Important dates: nestbuilding began 3 Dec, nest complete 7 Dec, copulations 7-11 Dec, 1st egg laid 12th Dec, 5th egg laid 16th Dec, started incubating 15th Dec, chicks hatched 30/31 Dec (5th egg died at hatch), 1st shedding by chicks 9 Jan

Table 1: Pre-laying to post-hatching oocyst shedding by parents of 1997-98 clutch

All results are shown as number of oocysts per sample

Date	No. of oocysts from dam ("Jill")	No. of oocysts from sire ("Ray")
18 Nov	0	0
25 Nov	0	0
3 Dec	0	0
8 Dec	0	0
16 Dec	NS	0
23 Dec	NS	0
30 Dec	0	NS
5 Jan	0	NS

NS = no sample

1998-1999 season

1st clutch

Important dates: nestbuilding began 30 Oct, nest complete 10 Nov, copulations 10-15 Nov, egg laying complete 15 Nov, started incubating 16 Nov, chicks hatched 1 Dec 1998, 1st shedding by chicks 23 Jan

Table 2: Pre-laying to post-hatching oocyst shedding by parents of 1998-99 1st clutch

All results are shown as number of oocysts per sample (weight of faeces in grams)

Date	No. of oocysts from dam ("Jill")	No. of oocysts from sire ("Ray")
13 Oct	0 (0.2)	0 (0.48)
27 Oct	0 (0.24)	0 (0.12)
30 Oct-2 Nov	0 (0.65)	0 (0.17)
4-6 Nov	0 (0.29)	0 (0.42)
9 Nov	0 (0.5)	0 (0.4)
10-11 Nov	0 (0.95)	0 (0.29)
12 Nov	0 (0.3)	0 (0.11)
14-16 Nov	0 (1.25)	0 (0.66)
24 Nov	0 (w)	0 (w)
27 Nov	NS	0 (w)
4 Dec	NS	0 (w)

w = weight not recorded

2nd clutch

Important dates: nestbuilding began 21 Jan, nest nearly complete 27 Jan, 1st egg laid 31 Jan, chicks hatched 17-18 Feb 1999, 1st shedding by one of chicks 3 March

Table 3: Pre-laying to post-hatching oocyst shedding by parents of 1998-99 2nd clutch

All results are shown as number of oocysts per sample (weight of faeces in grams)

Date	No. of oocysts from dam ("Jill")	No. of oocysts from sire ("Ngatiwai")
9 Jan	0 (0.08)[a]	NS
10 Jan	0 (0.03)	NS
11 Jan	0 (0.04)[a]	NS
12 Jan	0 (0.17)	NS
13 Jan	0 (0.12)	0 (0.18)
20 Jan	0 (0.12)	0 (0.09)
26 Jan	0 (0.21)	0 (0.18)
2 Feb	0 (0.33)	0 (1.24)
16 Feb	NS	0 (0.85)
23 Feb	NS	0 (0.44)

[a] = sample pooled with chicks
NS = no sample

APPENDIX VI

Results of faecal examinations of hihi samples from islands

Table 1: Results of faecal examinations of hihi samples from Mokoia Island

Sample Date	Source (nest or bird)	No. of oocysts per sample
Jan 23 1998	VSG nestlings	0
	VSG 291	0
	VSG 290	0
	BG/B/288	0
	VSG 290	0
	Keith	0
	BG/B/295	0
	adult F AB/BW VSG	0
	fledgling	1133 oocysts[a] (some sporulating)
Jan 27 1998	QSG	600
	RB/AG 289 BGB	0
	BGB 295 A/YR	0
	290 VSG WW/WA	0
	pooled sample BGB	1 only
	RRRA VSG	0
	288 BGB A/B	0
	W?/WA 190 Y?69	0
	288 BGB A/B	0
Feb 4 1998	BGB Nestcup	>1000
	RA/B	0
	A/BB juvenile	0
	52296 GA/G	0
Nov/Dec 1998	nest 36 (3X13 day old chicks)	0
	nest 59 (2X22 day old chicks)	0
Jan 29 1999	K Gully (1 wk old chicks)	0
	PTG (2 wk old chick)	0
Feb 1 1999	Site 59 (3 wk old chicks)	0

[a] = oocyst characteristics recorded in Appendix VII

Table 2: Results of faecal examinations of hihi from Tiritiri Matangi Island

Sample Date	Source (nest or feeder)	No. of oocysts per sample
Jan 1999	nestbox bush 21/2	110[a]
	nestbox	118[a]
	feeder YM/RG	0
	feeder RM	6
	feeder WM-YM	0
	feeder WM-YG	0
	nestbox	0
	feeder -/WM	0
Feb 1999	B 1/8	48
	B 1 feeder	30
	unrecorded number of other samples	0
	nestboxes: B 1/12	98
	B 1/18	0
	B 1/8	0
	B 1/14	0
	B 1/20	0
	Pa/4	0
Aug 1999	B 21/4	0[b]
	Box 10, Wattle Track	0[b]
	B24	0[b]
	B2/1	0[b]
	B2/2	0[b]
	Unidentifiable sample	0[b]
	Unmarked sample	0[b]
	B23/1	0[b]
	Bush 22, Box 6	0
	Bush 22, Box 14	0
	B 22/9	0
	Pa 4	low numbers
	B 1/20	moderate numbers
	B 1/18	0
	B 22/10	very small numbers of sporulated oocysts
	B 22/4	very small numbers
	B 22/2	0
	B 22/8	0
	B 22/12	0
	B 22/1	0
	B 1/12	small numbers
	B 22/16	very small numbers

[a] = oocyst characteristics recorded in Appendix VII
[b] = estimated via faecal smear

APPENDIX VII

Morphological data on hihi oocysts

Table 1: Morphological characteristics of coccidia species from hihi at the N.W.C.

Source of oocysts	Oocyst type	Micro pylar cap	Oocyst size (µm) & shape	Micropyle distinct or indistinct	No. of polar granules	Sporocyst size (µm) & shape	Sporocyst residuum	Stieda body	No. of sporozoites
Jill & chicks, Feb-March 1998	A	N	round & regular 21.2x21.6	indistinct	1?	ovoid? 15.52x10.24 15.36x10.24	?	?	2 sporocysts, 4 sporozoites in each
	A	N	round & regular 22.08x20.96	indistinct	0	ovoid? 18.16x10.8 17.84x9.6	Y	?	2 sporocysts, 4 sporozoites in 1, only 2 discernible in other
Ngatiwai, May 1999	A	N	round 21.28x20.88	indistinct	0	1 ellipsoid, 1round 110.64x10.56 13.92x9.52	Y	Y	2 sporocysts, 4 sporozoites in each
	A	N	round 19.6x17.76	indistinct	1?	ovoid 16.16x8.72 15.76x8.72	Y	Y	2 sporocysts, 4 sporozoites in each
	A	?	almost round 19.68x17.76	indistinct	1?	1 ovoid, 1 almost round 12.8x9.28 9.84x8.8	Y	Y	2 sporocysts, 4 sporozoites in 1 and other sporocyst incompletely sporulated
	B	N	ellipsoid 18.8x15.44	indistinct	1	1 almost round, 1 ovoid 8.24x7.52 12x8.72?	?	Y	incompletely sporulated
	A	N	almost round 17.76x16.96	indistinct	0?	1 almost round, 1 ellipsoid 9.84x8.16 11.36x9.6	?	?	incompletely sporulated
	A	N	almost round 19.12x16.8	indistinct	1?	2 rather amorphous sporocysts, incompletely sporulated (degenerating?)			incompletely sporulated

Source of oocysts	Oocyst type	Micro pylar cap	Oocyst size (μm) & shape	Micropyle distinct or indistinct	No. of polar granules	Sporocyst size (μm) & shape	Sporocyst residuum	Stieda body	No. of sporozoites
Ngatiwai, May 1999	A	N	round 19.76x18.4	indistinct	1?	round 10x9.52 10.08x10.08	?	N	2 sporocysts, 4 sporozoites in each
	A	N	almost round 16.4x15.6	indistinct	N	not completely formed			
	A	N	almost round 19.6x18.32	indistinct	1	not completely formed			
	A	N	almost round 19.84x19.12	indistinct	1	not completely formed but look approx. round			
	A	N	round 18.48x18.48	indistinct	1?	Incompletely formed			
	B	N	ellipsoid 21.68x18.32	indistinct	1?	Incompletely formed but both look round-ellipsoid 12.64x8.96 8.64x8.16			
	A	N	almost round 17.04x15.36	indistinct	?	incompletely formed			

Y = present; N = absent; ? = uncertain

Table 2: Morphological characteristics of coccidia species from hihi on Mokoia Island

Oocyst type	Micro pylar cap	Oocyst size (μm) & shape	Micropyle distinct or indistinct	No. of polar granules	Sporocyst size (μm) & shape	Sporocyst residuum	Stieda body	No. of sporozoites
B	N	ellipsoid 25.28x20.64	indistinct	0	bimorphic ellipsoid 21.36x10.56 16.8x11.28	?	Y	2 sporocysts, 4 sporozoites in each
A	N	almost round 18.72x17.76	indistinct	0	ellipsoid 13.92x7.76 14.8x7.92	?	Y	2 sporocysts, 4 sporozoites in each
A	N	round 21.6x21.12	indistinct	?	ellipsoid 16.08x10.08 13.92x9.04	?	undetectable	2 sporocysts, 4 sporozoites in each
B	N	ellipsoid 26.56x21.12	indistinct	1	ellipsoid 16.64x14.32 13.6x9.84	?	Y	2 sporocysts, 4 sporozoites in each
Big	N	almost round 28.4x26.72	indistinct	0	ellipsoid 20.32x12 21.44x11.2	?	Y	2 sporocysts , 4 sporozoites in each
B	N	ellipsoid 24.64x21.36	indistinct	0	ellipsoid 13.76x8.64 18.72x9.28	?	Y	2 sporocysts, 4 sporozoites in each
Big	N	round 27.6x25.04	indistinct	1?	ellipsoid 20.24x12.48 18x11.44	2 obvious	Y	2 sporocysts, 2-4 sporozoites in each
A	N	almost round 17.52x15.92	indistinct	?	ellipsoid 14x7.52 13.92x7.84	?	Y	2 sporocysts, 4 sporozoites in each

N = absent; Y = present; ? = uncertain
Note: the oocysts were collected in January 1998

Table 3: Morphological characteristics of coccidia species from hihi on Tiritiri Matangi Island

Oocyst type	Micro pylar cap	Oocyst size (µm) & shape	Micropyle distinct or indistinct	No. of polar granules	Sporocyst size (µm) & shape	Sporocyst residuum	Stieda body	No. of sporozoites
A	N	round-ellipsoid 20.64x17.76	indistinct	?	ellipsoid 17.2x8.56 16.16x9.04	N	Y	2 sporocysts, 4 sporozoites in each
A	N	round 19.84x17.12	indistinct	?	ellipsoid 16.32x9.2 17.12x7.12	?	Y	2 sporocysts, maybe 4 sporozoites in each
A	N	round 19.2x19.12	indistinct	?	almost round 11.28x8.88 10.08x9.44	Y	N	2 sporocysts, maybe 4 sporozoites in each
A	N	almost round 20.32x18.16	indistinct	?	ellipsoid 13.44x9.36 12x8.72	Y	Y	2 sporocysts, maybe 4 sporozoites in each
A	N	almost round 22.96x20.32	indistinct	1 definite	not completely sporulated ellipsoid 12.96x9.28 12.96x9.52	?	Y	2 sporocysts, no sporozoites completely formed
B	Y	ellipsoid 24.48x20	distinct	nil	round-ovoid 12.72x10.48 10.32x10.16	Y	Y	2 sporocysts, maybe 4 sporozoites in each
A	N	round 21.76x21.04	indistinct	1 definite	ellipsoid 12.4x12 14.4x9.84	Y	N	2 sporocysts, maybe 4 sporozoites in each
A	N	almost round 22.64x22.08	indistinct	nil	1 round, 1 distorted round 11.12x10.24 13.12x11.84	Y	N	2 sporocysts, incompletely sporulated
B	N	elllipsoid 24.48x20.88	indistinct	nil	ovoid 17.52x9.28 19.84x10.8	?	Y	2 sporocysts, 4 sporozoites in each
B	N	ellipsoid 26x18.08	distinct	nil	1 ellipsoid, 1 ovoid 16.48x11.52 12.32x10.72	Y	Y	2 sporocysts, maybe 4 sporozoites in each
A	N	round 20.48x20.16	indistinct	1	round 11.28x11.28 11.36x10.64	?	N	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 25.2x18.56	indistinct	nil	ovoid 19.84x9.6 19.28x9.92	?	?	2 sporocysts, no. sporozoites not discernible
A	N	round 21.36x19.76	indistinct	?	ellipsoid 15.2x10.48 15.84x9.28	?	?	2 sporocysts, no.sporozoites not discernible
B	N	ellipsoid 23.04x19.92	indistinct	?	ovoid 20.48x12.4 20.32x11.2	?	Y	2 sporocysts, no. sporozoites not discernible

Oocyst type	Micro pylar cap	Oocyst size (μm) & shape	Micropyle distinct or indistinct	No. of polar granules	Sporocyst size (μm) & shape	Sporocyst residuum	Stieda body	No. of sporozoites
B	Y	ellipsoid 24.24x19.36	distinct	1?	ovoid 17.12x9.28 13.68x8.64	Y	?	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 23.2x19.44	indistinct	?	round 13.12x11.76 14.08x11.68	Y	N	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 22.32x18.56	indistinct	?	ellipsoid 18.8x9.68 17.92x10.48	?	?	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 23.52x18.4	indistinct	?	almost round 11.12x9.68 11.76x11.52	Y	N	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 26.88x20.8	indistinct	?	ovoid 19.84x12 11.44x8.4	?	Y	2 sporocysts, no.sporozoites not discernible
B	N	ellipsoid 24.48x17.68	indistinct	?	ovoid 15.44x10.48 17.6x9.44	Y	Y	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 28.4x20.56	indistinct	1	1 ovoid, 1 ellipsoid 14.88x10.48 18.72x10.48	?	Y	2 sporocysts, no. sporozoites not discernible
B	?	ellipsoid 26.72x21.36	indistinct	1	almost round, sporocysts appear to have "eviscerated"	?	N	2 sporocysts, no. sporozoites not discernible
A	N	round-ellipsoid 21.52x18.64	indistinct	nil	ovoid 16.96x10.4 17.2x11.6	?	Y	2 sporocysts, no. sporozoites not discernible
B	N	slightly ovoid 23.52x20	indistinct	?	ovoid 15.2x10.64 12.4x9.84	Y	?	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 24.32x21.84	indistinct	1	1 almost round, 1 ovoid 19.92x11.68 15.2x12.56	Y	?	2 sporocysts, no. sporozoites not discernible
B	N	ellipsoid 25.92x19.84	distinct	nil	ovoid 16.88x10.4 17.28x11.76	?	Y	2 sporocysts, no. sporozoites not discernible

N = absent; Y = present; ? = uncertain

APPENDIX VIII

Necropsy procedure:

a) Duplicate blood smears were made from clipped toenails.

b) An external examination of the body was made noting body condition, feather and skin condition, the nature of the faecal and urate material, and any lesions, discharges or any abnormalities.

c) The feathers were dampened down with dilute Teepol solution and a further external examination made.

d) The bird was positioned in dorsal recumbancy and a midline skin incision made from the base of the bill to the tip of the cloaca.

e) The skin was retracted and the subcutaneous tissues dissected. The bill was incised down the middle and the oral cavity examined.

f) The abdominal muscles were opened exposing the abdominal viscera, which were examined *in situ.*

g) The sternum was removed after cutting through the ribs laterally and through the corocoids and clavicles. The thoracic viscera were examined *in situ.*

h) The entire gastrointestinal tract was removed from the tongue to the cloaca.

i) All remaining viscera were removed.

j) The brain was removed.

k) One or two joints were examined by incising into them and exposing the tissues.

l) The remaining carcass was examined for any abnormalities.

m) The gastrointestinal tract was opened along its entire length. The presence of any parasites and other abnormalities were noted. The tract was stapled onto cardboard to minimize shrinkage and curling.

n) All other viscera were examined and incised.

o) All tissues, and the remaining carcass, were fixed in 10% buffered formalin.

APPENDIX IX

Results from transmission experiments.

(i) Results of Hihi Necropsy

Gross necropsy findings: The bird weighed 30.39 g and was in moderate-good body condition. There was no feathering from the thoracic inlet to the abdomen on the ventral surface. The abdomen was swollen and contained up to 2 ml of red fluid. There was yellowing of the subcutaneous abdominal tissues. The liver was enlarged, pale tan, with multifocal single and coalescing cream-yellow spots up to 1 mm in diameter over the surface and throughout the parenchyma.

The airsacs and the pericardial sac were moderately gelatinous. There was a haemorrhage measuring approximately 7 x 4 mm on the epicardium. The lungs were congested and contained dark red areas of consolidation.

The peritoneal wall was red with multiple large plaques of pale yellow material on its surface. There was a solid yellow mass measuring 5 mm diameter free in the abdomen.

A 15 mm length of the caudal intestine was a deep yellow on its serosal surface. The remaining intestine was very red on its serosal surface and intensely red on its mucosal surface.

The bursa was enlarged at 10 x 7 mm and its serosal surface was a blotchy yellow and red.

A "starburst" crystalline pattern was visible on the surface of the kidneys.

Segments of intestine were taken every 5-10 mm for histology.

Histopathology: The liver contained multiple and coalescing foci of inflammatory necrosis infiltrated centrally by mononuclear inflammatory cells. In the centre of these areas and also within and around the outside of many large hepatic blood vessels, were numerous branching septate hyphae. There was a moderate amount of haemosiderin (confirmed by a Perl's stain) within hepatocytes and within Kupffer cells.

The lung also contained multiple inflammatory foci in which were numerous fungal hyphae mixed with necrotic debris and inflammatory cells. Some of these foci were partially contained within a peripheral layer of fibroblasts and in others the hyphae were spreading actively into surrounding lung parenchyma. In the centre of some of these foci were dense eosinophilic foci of necrosis. There were also fungal hyphae

present in many pulmonary blood vessels. Surrounding the bursa there was a severe extensive peritonitis involving large numbers of fungal hyphae, lymphocytes and macrophages, mixed with necrotic debris. The infection also involved the entire bursa with loss of normal architecture, destruction of bursal follicles and depleted lymphoid tissue.

Throughout the gastrointestinal tract there was a severe granulomatous peritonitis in which large numbers of similar hyphae and inflammatory cells were present. The hyphae extended into the gizzard muscle. There was a milder peritonitis, also associated with hyphae, surrounding the spleen.

There was an area of fungal hyphae invasion and inflammatory cell infiltration around the epicardium, and these elements were extending into the myocardium. Fungal hyphae were present in enlarged blood vessels beneath the epicardium and within the myocardium at this point.

There were no significant findings in the kidney, ovary, pectoral muscle or leg muscle.

Microbiology: Liver- Moderate growth of *Aspergillus fumigatus;* Lung- Light growth of *Aspergillus fumigatus*; Kidney- Light growth of *Aspergillus fumigatus*; Duodenum- No *Salmonella* or *Yersinia* isolated.

Diagnosis: Chronic active mycotic pneumonia, hepatitis, bursitis, myocarditis and peritonitis due to *Aspergillus fumigatus.*

(ii) Results of Finch Transmission Experiment

Table 1: Results of finch faecal examinations

All results are shown as number of oocysts per sample (weight of sample in grams)

Treatment/Activity	Date	Control Group	Inoculated Group
pre-treatment	Feb 23	0	same as Control; group as one
	24	some Isospora-type oocysts, many already sporulated	(as above)
	25	(as above)	(as above)
	26	(as above)	(as above)
toltrazuril treatment	March 4		
(as above)	5		
(as above)	6		
	7		
	8		
	9		
	10		
toltrazuril treatment of additional birds	11		
(as above)	12	0	0
(as above)	13		
	14	0	0
	15	0	0
euthanasia of 2 Control birds. inoculation	16	no faeces collected	no faeces collected
	17	0	0
	18	0	0
	19	0 (1.47)	0 (1.61)
	20	0 (1.61)	0 (1.49)
euthanasia of 3 birds	21	0 (1.42)	0 (1.36)
	22	0 (0.76)	
	23	0 (0.88)	0 (1.01)
	24	0 (1.25)	0 (1.62)
	25	0 (0.71)	0 (1.22)
euthanasia of 3 birds	26	0 (1.19)	0 (0.86)
	27	0 (1.35)	0 (1.55)
	28	0 (0.65)	0 (1.33)
	29	0 (0.63)	0 (1.06)
	30	0 (0.90)	0 (1.50)
euthanasia of 3 birds	31	0 (0.18)	0 (0.62)
	April 1	0 (0.29)	0 (1)
	2	0 (0.65)	0 (0.88)
	3	0 (0.65)	0 (0.76)
	4	0 (0.36)	0 (0.89)
euthanasia of 3 birds	5	0 (0.59)	0 (0.87)

Table 2: Worksheet used in the recording of histopathological findings for finches (case number 30147 given as example)

	Inflammatory cells	Haemosiderin	Haemorrhage	Fatty infiltration	Fungi	Other eg coccidia
Brain	-	-	-	-	-	-
Heart	-	-	-	-	-	-
Lungs	-	-	-	-	-	-
Oesophagus & Crop	NE	NE	NE	NE	NE	NE
Proventriculus	+	-	-	-	-	-
Gizzard	+	-	-	-	-	-
Bursa	NE	NE	NE	NE	NE	NE
Liver	severe, multifocal mixed	-	-	-	-	-
Spleen	-	-	+	-	-	-
Kidney	+	-	-	-	-	-
Pectoral muscle	-	-	-	-	-	-

Intestine	Lymphocytes	Plasma cells	Granulocytes	Macrophages	Epithelium	Coccidia
Proximal	+	-	-	-	-	-
Mid 1	+	-	-	-	-	-
Mid 2	+	-	-	-	-	-
Distal	+	-	-	-	-	-

Fat reserves: minimum (scale- minimum, moderate, good)

mild= +
moderate= ++
severe= +++
very severe= ++++
epithelium: hyperplasia, necrosis, intraepithelial inflammatory cells
NE= not examined