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# ASPERGILLOSIS AND OTHER CAUSES OF MORTALITY IN THE STITCHBIRD IN NEW ZEALAND

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ABSTRACT: Necropsy findings from natural deaths in free living and captive stitchbirds (*Notiomystis cincta*) were examined over a 3 yr period (November 1991–94) to establish whether disease was an important factor in translocation failures and captive breeding programs undertaken by the New Zealand Department of Conservation. Fresh and fixed material from seven free-living birds and 11 captive birds were examined and were compared with those of a retrospective study of archival material from captive and wild birds collected over a 13 yr period (1979–91). The causes of death in both the present and retrospective study showed a similar pattern with aspergillosis and aspiration pneumonia being the most significant cause of mortality in captive birds. Aspergillosis was diagnosed as the cause of death in 11 of 31 stitchbirds from Mt Bruce; eight of these deaths occurred in the winter months (June–August). The other causes of death in captive birds included trauma, coccidiosis, and sporadic bacterial infections. Hemosiderosis and airsacculitis were common histological findings in most of the wild and captive stitchbirds examined.

*Key words:* Aspergillosis, diseases, mortality, *Notiomystis cincta,* stitchbird.

## INTRODUCTION

The stitchbird or hihi (*Notiomystis cincta*), is a member of the Meliphagidae which includes the bell bird (*Anthornis melanura*), the tui (*Prosthemadura novaeseelandiae*), and the red wattle bird (*Anthochaera canunculata canunculata*). The former three species are native to New Zealand while the latter is more common in Australia and only occasionally is seen in New Zealand.

Unlike the tui and the bell bird the stitchbird is no longer widely distributed. The main population, estimated to be between 4,000 and 5,000 individuals is presently restricted to Little Barrier Island (36°15′S, 175°30′E) although some birds have been transferred to Hen, Cuvier and Kapiti Islands (Angehr, 1985). Attempts to set up a free-living population on Kapiti Island (40°55′S, 174°50′E) have had limited success. However, a small breeding group has been established at the New Zealand National Wildlife Centre (Mt Bruce, New Zealand; 40°30′S, 174°30′E). This group has been carefully monitored over the past 15 yr with routine necropsies performed on all dead birds. The following report describes the necropsy findings in 31 captive stitchbirds submitted for necropsy by the National Wildlife Centre captive breeding program, and seven were submitted from Kapiti Island by conservation officers undertaking a translocation program on the island.

Few reports of necropsy findings in the Meliphagidae are available and none have been previously published on causes of mortality in the stitchbird. It has been reported that aspergillosis has been a significant cause of mortality in the helmeted honey eater (*Linchenostomus melanops cavidix*) and is possibly an important factor limiting the success of captive breeding programs in this and other species (Smales et al., 1991). The current and retrospective studies were undertaken in order to establish whether disease was an important factor in translocation failures and in the Mt Bruce captive breeding program.

## MATERIALS AND METHODS

Necropsy findings from 38 stitchbirds were examined over a 15 yr period (1979–94). Cases submitted for necropsy during the last 3 yr of this period were part of a specific avian disease investigation project conducted by the New Zealand Department of Conservation (Wellington, New Zealand) and Massey University (Pal-

merston North, New Zealand). These cases have been considered separately due to the fact that the protocols used for the disease investigation study differ from the routine necropsy procedures carried out prior to 1991. The protocols used were devised to screen for disease problems suspected from results obtained from retrospective studies (Cork et al., 1995).

Fresh and/or fixed material was collected from naturally dead captive and free-living stitchbirds over a 3 yr period (November 1991–94) and submitted to the Department of Veterinary Pathology and Public Health (Massey University) for necropsy. Eleven captive birds were submitted from the National Wildlife Centre, and seven free-living birds were submitted from Kapiti Island. Where possible, fresh tissues were collected for microbiological examination (lung, liver, kidney, spleen and intestinal contents). Sections of lung and air sac were collected for culture of *Aspergillus* spp. on Sabouraud dextrose agar (Difco Co., Detroit, Michigan, USA) and incubated at 27 C. If indicated, on clinical grounds or gross findings, sections of other tissues were cultured for *Staphylococcus* spp. *Streptococcus* spp. *Salmonella* spp. *Escherichia coli, Yersinia* spp. and *Pasteurella* spp. using standard culture techniques with incubations at 37 C on sheep blood agar and MacConkey agar (Difco Co.). *Yersinia pseudotuberculosis* was grown on Cefsulodin-Irgasan-Novobiocin agar (Difco Co.) at 28 C and identified by the methods outlined by Carter (1982). Fecal flotation and examination of intestinal contents for helminth eggs and coccidia was performed using standard techniques (Sloss and Kemp, 1978). Where carcasses could not be submitted fresh the whole body of the bird was immersed in 10% buffered formalin after making an abdominal incision to allow rapid fixation of the viscera. Gross findings and, where available, clinical history were recorded for each bird submitted for histology. Tissues were processed routinely and embedded in paraffin. Sections were cut at 5 μm and serial sections from each block were stained with haematoxylin and eosin (Gill et al., 1974); Gram-Twort (Twort, 1924) and Perls' Prussian blue stain for ferric iron (Perls, 1867). Sections of lung, liver, and spleen also were stained with Periodic acid-Schiff (PAS) (McManus, 1946) and Young's fungal stain (Young, 1968).

In the retrospective study, paraffin embedded sections and gross findings recorded from 20 stitchbirds submitted to Batchelar Animal Health Laboratory (Palmerston North, New Zealand) from 1979–91 were examined along with case reports and, where available, microbiology reports. In some cases additional staining procedures were performed on cut sections (Cork et al., 1995). The necropsy procedures used for these cases followed routine veterinary necropsy guidelines. It should be noted that in some cases the carcasses of wild birds presented for necropsy prior to 1991 had been too decomposed for detailed examination. To avoid loss of valuable material, where immediate submission to a laboratory was not possible, the field staff of the Department of Conservation were instructed to conduct a brief necropsy and to open and fix specimens shortly after death.

## RESULTS

Thirty-eight stitchbirds were examined over a 15 yr period (1979–94). These included 16 adult and three juvenile females, 15 adult males and four chicks (undetermined sex). Thirty-one birds came from the captive breeding programe at the National Wildlife Centre and the remaining seven birds came from a free-living population on Kapiti Island which was established as part of a translocation program.

Of the 31 birds examined from the captive population at Mt Bruce, 11 birds had gross and/or histological evidence of mycotic pneumonia. *Aspergillus* spp. was cultured from two of these birds. In the other 9 birds, the demonstration of septate, branching, PAS positive fungal hyphae in the lungs and airsacs indicated a mycotic infiltration which was similar to that seen in aspergillosis. These cases were from the retrospective study or from fixed carcasses where culture could not been undertaken. Eight of the eleven cases of mycotic pneumonia occurred in the winter months (June–August), of these cases, four were adult males, two were adult females, and two were juvenile females. Ten of these birds had shown clinical signs of respiratory distress and/or clicking noises prior to death. In the eleventh bird the initial presenting signs were neurologic with torticollis, head tilt and disorientation over a period of 10 days. Following treatment with antibiotics and later nebulisation with antifungal drugs (Amphotericin B, Squibb, Hounslow, UK) the bird had died despite making an initial recovery. Necropsy of the bird revealed extensive yellow granulomatous lesions in the liver, lungs, and

brain. Histologically there was an airsaculitis with plaques along the inner wall of the thoracic and peritoneal airscas and a few small granulomas in the lung. Similar lesions were seen in the liver and brain. The remaining three cases of aspergillosis occured sporadically in the summer and autumn. Two other birds submitted from Mt Bruce were thought to have died following asphyxiation due to the lodgement of whole corn in the pharynx. In another bird there was a bee sting located in the distal oesophagus, this was surrounded by edema and inflammatory change around the thoracic inlet which was thought to have resulted in airway obstruction. Hepatic hemosiderosis was the only significant finding noted in five of 20 birds in the retrospective study. This also was a common finding in the stitchbirds which died of other causes in the retrospective and current studies. In many birds there was significant stainable iron in the Kupffer cells of the liver and also in hepatocytes. In one stitchbird there also was stainable iron in the spleen and in renal cortical cells. This was a 6-yr-old male bird which had histological evidence of severe glomerulonephritis and several adult *Capillaira* sp. in the intestine.

Coccidiosis was considered to have been the cause of death in one 2-mo-old stitchbird. The chick weighed 28 g and was found dead in the nest without any history of clinical disease. On gross examination of the bird the intestinal tract occupied the majority of the abdominal cavitiy and there was extensive thickening of the intestinal wall, numerous foci of necrosis scattered throughout the liver parenchyma and the spleen was enlarged. Histologically, numerous coccidia were seen in the lamina propria of the ileum, jejunum, and colon. There was significant destruction of epithelial cells with numerous coccidia in the glandular crypts of the intestine. All stages of the life cycle were represented in the intestinal tissue; some macrogamonts also were seen in the liver and numerous schizonts were observed in the spleen. Coccidial oocysts were later found in the intestinal lumen and tissues of the dead bird, in fecal material taken from the nest and in fecal samples taken from the parent birds housed in the same aviary. The oocysts had the appearance of those of the genus *Isospora.* There also were sections of *Capillaria* spp. in the intestinal crypts and some disintegrating remnants of nematodes in the liver parenchyma.

Bacterial septicaemia was responsible for the death of 5 captive stitchbirds. Two birds died following systemic infection with *Yersinia pseudotuberculosis.* Both birds died during the spring (September) following a period of cold weather, but in different years. *Yersinia pseudotuberculosis* was cultured from lesions in the liver, lung and spleen. In one bird there also were necrotic foci in the intestine. *Staphylococcus aureus*, *Streptococcus* spp., and *Echerichia coli* were cultured from visceral lesions in three other birds.

Of the remaining six birds submitted from Mt Bruce, one bird died following severe ingluveitis; no other lesions were detected at necropsy. One juvenile bird died suddenly following normal development; the only lesion detected was an area of vascularisation and fibrous change in the apical tip of the ventricles. The lesion occupied about 30% of the myocardial tissue and the rest of the heart musculature appeared histologically normal. The remaining three stitchbirds died following traumatic injury due to flying against the mesh of the aviaries; in each case this was attributed to the presence of the birds of prey above and near the aviary complex.

Birds submitted from the free-living Kapiti population included four adults (2 males and 2 females) and three chicks (undetermined sex). The three chicks had all died in a nest box over a period of 2 wk (October). This was thought to have been associated with a period of unseasonally cold weather which had occurred during this period. Other tree-nesting species also lost chicks during this period (I. Castro, unpubl. data); unfortunately these chicks

were too decomposed to be examined in detail.

Of the four adult birds examined, three birds had histological evidence of airsacculitis. Aspiration pneumonia was diagnosed as the cause of death in two of these birds, both adult males. The cause of death was not immediately clear on gross findings but histologically there was a slight thickening of the air sac wall with some inflammatory exudate present between the epithelial layers. There were foreign body multinucleate giant cells in some sections of the air sacs especially near secondary bronchi. In the bronchi there also was some black granular material. This did not stain with PAS as would be expected with *Aspergillus* spp., nor was it possible to culture *Aspergillus* spp. from the lung or air sac material. Both of these birds had a history of making excessive respiratory noises, although they had remained active. The third bird had been reported to be making clicking noises 1 wk prior to being found dead. In this bird there were unidentified mites seen in a section of the larynx but this may have been due to contamination. Mites were frequently found on the feathers and in the nest boxes of free-living and captive fledgling chicks. The actual cause of death in this third bird, a female, was thought to have been due to egg peritonitis with secondary complications due to opportunistic pathogens. *Echerichia coli* was cultured from the ruptured uterus and abdominal cavity. The fourth free-living bird had died following a prolonged history of arthritis. At necropsy *Staphylococcus aureus* was cultured from an old foot lesion and an inflamed joint. There was gross evidence of chronic inflammation in the affected joint. Histologically, there also was arteritis and inflammatory exudate in the peritoneal cavity.

## DISCUSSION

The high prevalence of aspergillosis in the stitchbirds at the National Wildlife Centre over the past 15 yr poses some questions about the management of the captive population. *Aspergillus* sp. fungi are generally considered to be opportunistic pathogens which tend to flourish in damp leaf litter (Carter, 1982). Mt Bruce is situated in a cold area, surrounded by native podocarp forest and fern glades, in the lower North Island of New Zealand. The aviaries and the breeding boxes are well protected by vegetation which may restrict the amount of direct sun light into the enclosures. Meliphagidae and other birds confined for captive breeding programs, especially those on display to the public, are frequently under some form of physiological stress which may be greater at certain times of the year, such as during the breeding season or in the winter where environmental conditions are more severe. Such birds are more likely to develop a clinical infection following the inhalation of fungal spores (Wallach and Boever, 1983; Reece, 1989). Where a damp leaf litter environment has developed in an aviary, the growth of *Aspergillus* spp. and other fungi is encouraged. As a consequence, birds probably receive a repeated exposure to the organism. In the current study eight of 11 cases of mycotic pneumonia occurred in the winter months. Changes in management, such as regular clearing of nest box sites and opening up the canopy to increase the amount of sunlight reaching the floor of the aviaries, have been suggested. Aspergillosis was not diagnosed as a cause of death in free-living stitchbirds examined but this may reflect the early stage of the study. Most of the free-living birds had evidence of airsacculitis and some respiratory embarrassment, this may predispose to later development of mycotic infection if environmental factors favour the survival of the organisms.

Hepatic hemosiderosis was a common histological finding in both wild and captive stitchbirds. The significance of this remains unclear but the subject has been reviewed by Lowenstine and Petrak (1980). In many adult stitchbirds and in one 2-mo-old chick with concurrent infectious dis-

ease there was a high degree of Kupffer cell loading seen in Perls' iron stained sections. This may be due to increased turnover of red cells and/or body tissue due to the infection itself or due to pre-disposing factors such as starvation (Borch-Johnson and Neilson, 1987; Cork et al., 1995). The role of dietary iron in the development of the condition in birds is unclear, but in some species there appears to be a genetic predisposition to the development of hemosiderosis (Lowenstine and Petrak, 1980).

Coccidiosis was the cause of death in one chick. This bird had extensive lesions in the intestine, liver and spleen. In the case described herein the infection is particularly severe, indicating that the bird probably had a compromised immune system as suggested by the depleted lymphoid tissue of the spleen. The oocysts had the appearance of *Isospora* sp. but further work is currently underway to characterise the parasite. *Isospora* spp. are predominantly found in passerines, but although many species of *Isospora* have been listed, their clinical significance and life cycle has yet to be fully described (Todd, 1981). Unlike *Eimeria* spp., *Isospora* spp. are not considered to be totally host specific (Box, 1981; Keymer, 1982). Coccidial oocysts have been identified in fecal tissue taken from other New Zealand native passerines at the National Wildlife Centre and from wild birds including the kokako (*Callaeas wilsoni*) and saddleback (*Philesturnus carunculatus*) (S. C. Cork, unpubl. data); but it appears that these oocysts are not the same species as those described in the stitchbird.

It is difficult to determine the significance of the concurrent *Capillaria* sp. infection in the cases examined. There were numerous adult parasites in the lumen of the intestine of the chick but this was not thought to have been the cause of death. In an adult male bird which had died following acute glomerulonephritis, there was a low intensity of adult *Capillaria* sp. which was reported as an incidental finding.

Other causes of death outlined in the captive population of stitchbirds have occurred sporadically over a 15 yr period (1979–94). Most of these deaths were due to opportunistic pathogens or trauma.

The free-living population of stitchbirds has yet to become established on Kapiti and other offshore islands. It is too early to draw conclusions about the disease patterns in these birds. Continued monitoring of translocation programs by the Department of Conservation will be necessary to avoid future losses due to mycotic pneumonia in these birds during or recently following transfer. Stitchbirds appear to be susceptible to the development of aspiration pneumonia and subsequent airsacculitis which could be a predisposing factor to the development of mycotic disease. Since *Aspergillus* sp. are widely distributed in the environment, especially in places preferred for nesting by stitchbirds, control of environmental *Aspergillus* sp. is not very practical, it is important to identify and remove those factors which predispose to the development of mycotic disease. However, it would be advisable to pay some attention to regular clearing of nest boxes which also seem to serve as reservoirs for the build up of coccidia and mites. This study has outlined the common causes of death in captive and some free-living stitchbirds. Whereas it is only a preliminary report, it has identified some key health problems which has already resulted in a reevaluation of some management policies. The study has attempted to utilise a valuable but limited resource of necropsy material and archival records to assess the health status of stitchbirds in captive breeding programs and compare these with preliminary findings from free-living birds. The limitations of the data are appreciated, but this is a factor common to many studies on rare and endangered species where there is little control over the nature and quality of specimens received. It is hoped that the data and con-

clusions from this communication may serve as a reference point for future studies on the health of free-living and captive stitchbirds in an attempt to identify and reduce factors leading to mortality in this rare and unique species.

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