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Ventral dermatitis in rowi (*Apteryx rowi*) due to cutaneous larval migrans

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ABSTRACT

The rowi is a critically endangered species of kiwi. Young birds on a crèche island showed loss of feathers from the ventral abdomen and a scurfy dermatitis of the abdominal skin and vent margin. Histology of skin biopsies identified cutaneous larval migrans, which was shown by molecular sequencing to be possibly from a species of *Trichostrongylus* as a cause of ventral dermatitis and occasional ulcerative vent dermatitis. The predisposing factors that led to this disease are suspected to be the novel exposure of the rowi to parasites from seabirds or marine mammals due to the island crèche and the limited management of roost boxes. This is the first instance of cutaneous larval migrans to be recorded in birds. Severe and fatal complications of the investigation resulted in the death of eight birds of aspergillosis and pulmonary complications associated with the use of bark as a substrate in hospital. Another bird died of renal failure during the period of hospitalisation despite oral and intravenous fluid therapy. The initiating cause of the renal failure was not determined. These complications have the potential to undermine the working relationship between wildlife veterinarians and conservation managers. This case highlights that intensive conservation management can result in increased opportunities for novel routes of cross-species pathogen transmission.

1. Introduction

Rowi (*Apteryx rowi*), also known as Okarito brown kiwi were only recognised as a unique species of kiwi (*Apteryx* spp) in 2003 (Burbidge et al., 2003; Shepherd and Lambert, 2008). The Department of Conservation in New Zealand classifies the population as 'threatened: nationally critical' (Robertson et al., 2013), and by the IUCN Red List as endangered (BirdLife International, 2014). The total population was estimated at 375 birds in 2011. The geographic distribution of the wild population is confined to a single protected area of forest of approximately 11,000 hectares. This area is located northwest of Franz Josef/Waiau village on the west coast of the South Island of New Zealand.

The rowi population is intensively managed by the Department of Conservation (DOC). One component of the conservation management of the species is an Operation Nest Egg programme based on the principle that the highest mortality of kiwi due to mustelid predators (especially stoats *Mustela erminea*), occurs during the birds' early growth period. Eggs are removed from the burrows of wild birds, artificially incubated and hatched, and the young chicks are reared in predator free crèches. The young chicks are initially reared in indoor brooders, then placed in outside pens, and subsequently moved to a predator-free offshore island (crèche island) where they are largely independent and only monitored intermittently. Finally, they are returned to the wild population when sufficiently grown to reduce predation mortality (mean 361 days of age ± SD 106 days) (Colbourne et al., 2005).

In July 2013, a Department of Conservation ranger reported that on physical checks of the 30 young birds on the crèche island, 15 birds were showing loss of feathers from the ventral abdomen and around the vent and a crusty dermatitis of the abdominal skin and

Note: Nucleotide sequence data reported in this paper are available in the GenBank™ databases under the accession number KM434192.

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vent margin. One bird had an ulcerative dermatitis of the vent margin. The ranger reported that a mild version of this condition had been seen in three birds out of 30 in 2012, but was not further investigated and had spontaneously resolved. No similar condition had previously been seen in rowi of any other age group nor reported in other kiwi species.

In 2013, one bird with ulcerative dermatitis of the vent margin was removed from the crèche island for treatment at the same time as a bird that showed no signs of dermatitis but had sustained a traumatic leg injury and capture myopathy. The birds were admitted for diagnostic work up and treatment to The Nest Te Kōhanga veterinary hospital at Wellington Zoo. DOC rangers carried out a survey of all juvenile kiwi (18 birds) on the crèche island and found 14 more rowi with signs of dermatitis. After consultation with veterinarians at Wellington Zoo, the Wildbase Hospital at Massey University and the DOC wildlife veterinarian, these birds were captured for diagnostic investigation and treatment.

This paper describes the collaborative investigation of the ventral dermatitis of juvenile rowi and the fatal complications associated with the hospitalisation of the birds.

2. Materials and methods

As this was a veterinary diagnostic investigation, animal ethics approvals were not required. The handling, transport, diagnosis and management of the birds were approved by the Department of Conservation.

Birds were captured by hand from roost boxes and natural burrows on the island and transported by boat, car and ferry to The Nest Te Kōhanga veterinary hospital at Wellington Zoo. Seven birds were transported to the Wildbase Hospital at Massey University and the remaining seven birds were kept at The Nest Te Kōhanga, Wellington Zoo. The Nest Te Kōhanga already had two rowi hospitalised, one with an injury and one with dermatitis, so nine birds in total were held in this hospital. Both institutions followed an agreed diagnostic plan for the investigation of the dermatitis; however, there were differences in the husbandry and treatment of the birds at the different veterinary hospitals, that were largely due to different standard operating protocols for patient care.

Birds kept at Wildbase were housed in an indoor room with rubber matting covered by towels as substrate. Multiple roosting boxes were provided and food was offered in a communal tray. The birds were fed a captive kiwi diet consisting primarily of minced cow heart, vegetables, and a mineral supplement. Initially all the birds were housed together. Body weights were monitored daily. When three birds failed to maintain or gain weight, these birds were housed singly in stainless steel cages with rubber mat flooring covered by towels and with a roost box provided. These birds were hand-fed small meat patties of the kiwi captive diet twice daily until weights were increasing again.

Initially, the two rowi kept at the Nest Te Kōhanga (one with trauma and the other with dermatitis) were fed and monitored similarly to those at Wildbase. However, when the remaining birds arrived, all the birds kept at the Nest Te Kōhanga were housed in semi-outdoor enclosures, on a natural substrate comprised of shredded *Eucalyptus* bark. The birds were housed in one group of three and one group of four initially and each individual had a roost box in which the natural substrate was covered by towels. Later, the birds were housed separately and all the natural substrate was removed and replaced with rubber matting covered by towels as substrate. Birds that were unwell clinically were housed indoors individually in intensive care units with soft bedding covered by towels.

Given the unique presentation of the dermatitis, a broad diagnostic plan was implemented. Where a variation in the number of birds tested occurred this is noted. The diagnostic tests used included:

2.1. Physical examination

Full physical examinations were carried out on all birds (15/15 birds).

2.2. Faecal parasitology

Faecal floats using zinc sulphate as the concentrating fluid were examined at one laboratory by microscopy for evidence of gastrointestinal parasitism (15/15 birds). The birds at The Nest Te Kōhanga hospital were screened for *Cryptosporidium* spp. by acid-fast staining of faecal smears (8/15 birds).

2.3. Skin cytology

Cytology of affected vent margin was carried out using skin scrapes of the affected areas. Samples obtained in this manner were examined under oil for parasites and tissue smears were stained with modified Wright's stain (Diff-Quik) and assessed microscopically (14/15 birds). One bird died before ante-mortem skin scraping was performed.

2.4. Haematology and plasma biochemistry

Blood sampling was carried out for haematology and biochemistry in all birds 1–3 days after admission to hospital. The haematology was carried out by avian veterinarians at both hospitals using the same manual method of estimating white cell counts from blood smears that were stained with modified Wright's stain (Diff-Quik). The estimated white cell counts were performed on the blood smears following the method described in Fudge (2000). Quality control was achieved by one author (BG) reviewing all haematology slides. The parameters examined included packed cell volume, total plasma solids (g/L), estimated total white cell count, and the relative ratios and absolute counts for the leucocyte types identified in the peripheral blood smears, including heterophils, lymphocytes, and monocytes.

The plasma biochemistry panel included the following analytes: aspartate aminotransferase (AST); bile acids (BA); creatine kinase (CK); uric acid (UA); Glucose (Glu); calcium (Ca), phosphorus (P), sodium (Na), potassium (K), total plasma protein (TP), albumen (Alb) and total globulins (Glob) (15/15 birds). These plasma analytes were measured by the same point-of-care biochemical analyser (VetScan, Abaxis, Union City, CA, USA) at both hospitals.

2.5. Radiography

Full body radiographs using digital radiography were taken in two orthogonal views under general anaesthesia that was induced and maintained with inhalational isoflurane in oxygen (12/15 birds). Three birds died before radiographs could be carried out.

2.6. Enteric bacteriology

Cloacal swabs were taken from all the birds held at the Wildbase Hospital for aerobic bacterial cultures for the presence of *Salmonella* spp and *Yersinia* spp by a commercial veterinary diagnostic laboratory (7/15 birds). Due to laboratory error there are no results for cloacal microbiology from the birds held at The Nest Te Kōhanga.

2.7. Skin biopsies

Biopsies of the affected skin and vent margins were taken using 5 mm biopsy punches under general inhalation anaesthesia with isoflurane (8/15 birds). The remaining birds either died before sampling could occur or had shown significant improvement in their

dermatitis following anthelmintic treatment prior to biopsy. The skin biopsies were submitted for histological examination (8/8 samples), and for fungal and aerobic bacterial culture (6/8 samples) where sample volume allowed. Three skin biopsies were dissolved overnight in pepsin/HCl and the digested material examined with a dissecting microscope for the presence of larvae. Three mildly affected rowi that had not been previously sampled had skin biopsies four days after anthelmintic treatment for histological examination only.

2.8. Molecular diagnostics

2.8.1. PCR testing of skin scrapings for herpesvirus, poxvirus and Chlamydia

Skin scrapings of the lesions were examined by specific PCR testing for the presence of herpesvirus, poxvirus and *Chlamydia*. The methodology for these assays has been previously described; herpesvirus (Gartrell et al., 2009); avipoxvirus (Ha et al., 2013); and chlamydia (Gartrell et al., 2013) (10/15 birds). Not all birds at The Nest Te Kōhanga were sampled because of a miscommunication.

2.8.2. PCR testing of skin biopsies for nematode sequences

Tissue from five skin biopsies that were positive by histology for the presence of nematode larvae in the dermis were assessed by molecular techniques for nematode gene sequences. DNA was extracted from 10 µm sections of the five skin biopsy samples that were prepared for histological examination. The sections that were used for molecular analysis were from a sandwich cut, between two slides that showed nematodes histologically. DNA was extracted using a Qiagen DNeasy kit, following the manufacturer's instructions (Qiagen, CA, USA). PCR was performed to amplify the second internal transcribed spacer region (ITS-2) of the ribosomal (rDNA) region of the nematodes. Amplification of the nematode DNA was performed using the NC1-NC2 primer constructs (Gasser et al., 1993).

NC1: 5′-ACGTCTGGTTCAGGGTT-3′
NC2: 5′-TTAGTTTCTTTTCCTCCGCT-3′

For PCR amplification the conditions were as follows; 1 U Platinum Taq (Invitrogen, CA, USA), 1 X PCR buffer (200 mM Tris–HCl pH 8.4, 500 mM KCl), 1.5 mM MgCl2, 200 µM dNTPs each, 1 µM of each primer, 50 µg of template DNA in a total reaction volume of 25 µL. The reactions were carried out under the following conditions in a SensoQuest labcycler (Germany); 94 °C, 5 min, 94 °C, 30 s, 55 °C, 30 s, 72 °C, 30 s and for 40 cycles, 72 °C, 10 min. As positive controls for PCR, DNA previously extracted from a *Haemonchus contortus* nematode, and a clone of the *H. contortus* ITS-2 region were used. A negative PCR control of water was also utilised. An aliquot of 10 µL of the PCR product was separated on a 1% agarose gel that contained 0.2 mg·mL^{-1} of ethidium bromide for staining. Gels were run for 1 hour at a 100 V and were visualised with a UV transilluminator.

2.8.3. Sequencing of rDNA

Positive samples were then identified and the remaining 15 µL of the PCR product was cleaned using 70% ethanol and resuspended in elution buffer (10 mM Tris HCl, pH 8.0). The clean PCR product was sent to Massey Genome Service for Sanger sequencing in both directions. Paired sequence data were aligned using Geneious v 6.6.1 (http://www.geneious.com/). BLAST (Basic Local Alignment Search Tool (Altschul et al., 1990)) was used to compare the resulting sequences with published sequence data in NCBI (National Center for Biotechnology Information). PCR reactions and sequencing of the rowi samples were performed twice to confirm results.

2.8.4. Phylogenetic analysis

A total of 18 nematode species with published ITS 2 regions were selected for phylogenetic comparison to the material extracted from the rowi samples (hereafter called the rowi consensus samples). We included *Caenorhabditis elegans* as an outlier and model system. All sequence data were obtained from NCBI and when necessary trimmed to only the ITS 2 region using Geneious v 6.6.1. Utilising the tree building function of Geneious v 6.6.1, a distance matrix was constructed using the algorithm of Tamura and Nei (1993), and tree construction was performed using the Neighbor-Joining method (Saitou and Nei, 1987).

2.9. Post mortem examination

Birds that died (n = 10) during the investigation were subjected to a post mortem examination using standard veterinary protocols, and samples were taken for histological examination, and microbiological fungal and aerobic bacterial cultures.

2.10. Statistics

Multivariate GLM (SPSS v21) was used to assess any differences between the two hospitals for a range of quantitative measures including: bodyweights; haematology (packed cell volume, total plasma solids, estimated total white cell count, absolute counts for heterophils, lymphocytes, and monocytes); plasma biochemistry test results (plasma concentrations of AST, CK, UA, glucose, calcium, phosphorus, albumin, globulin, sodium and potassium); and faecal float results (coccidia, capillaria and strongyle egg counts). The overall significance of the model (Intercept*Hospital) was assessed using Pillai's trace. Kolmogorov–Smirnov and Shapiro–Wilk tests were used to examine the data for normality.

3. Results

3.1. Physical examination

Physical examination of the birds on admission showed a range of clinical abnormalities in the ventral abdominal and peri-cloacal skin (Fig. 1). All birds (15/15) showed alopecia and broken feather stubs in this area. Seven of fifteen birds showed evidence of mild dermatitis as characterised by hyperkeratotic scurfy skin. In three more severe cases there were small ulcers and fissures on the vent margin with varying degrees of exudation and matting of feathers to the vent.

3.2. Faecal parasitology

Faecal floats for parasitology detected a range of gastrointestinal parasites. Coccidial oocysts were present in 12/15 birds with a mean oocyst count/g of faeces of 27,197 (s.e. ± 12,364). *Capillaria* spp. nematode eggs were present in 7/15 birds with a mean count of 317 eggs per gram of faeces (s.e. ± 290), and strongyle-type nematode eggs were present in 6/15 birds with a mean count of 33 eggs per gram of faeces (s.e. ± 18.5). There was a significant difference ($F = 5.309$, $df = 3,11$, $p = 0.017$) in the quantitative parasite egg counts in rowi faeces from the two hospitals for coccidia, strongyle and capillaria type eggs (Table 1).

All 8 birds tested for *Cryptosporidia* were negative.

3.3. Skin cytology

Cytology of the skin surface was assessed in 14 birds. In 8/14 birds the cytology showed only normal epithelial squamous cells with free bacteria and no evidence of inflammation. In the remaining 6 birds there was evidence of acute dermal inflammation that

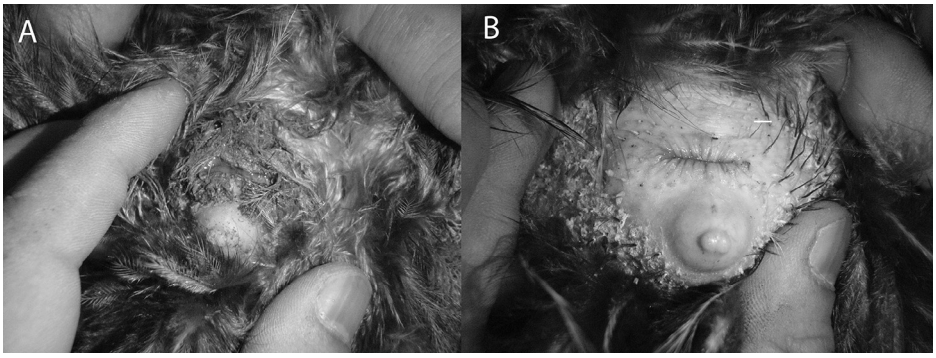

Fig. 1. The gross appearance at the ventral abdomen and vent of two rowi on initial capture on the crèche island. In bird A, there is exudative dermatitis matting the feathers to the vent and uropygial gland. In bird B, there is alopecia and broken feathers of the skin around the vent and hyperkeratosis of the surrounding skin.

was characterised by heterophils, eosinophils and abundant bacterial rods and cocci.

3.4. Haematology and plasma biochemistry

There was a significant difference ($F = 263.691$, $df = 7,5$, $p < 0.001$) in the haematology of the rowi kept in the two different veterinary hospitals (Table 2) taken in the first three days after hospitalisation, in particular the estimated white cell count (both with ($p = 0.001$) and without ($p < 0.001$) correction for PCV), and the absolute numbers of heterophils ($p = 0.001$) and monocytes ($p = 0.015$) were significantly higher in birds held at The Nest Te Kōhanga, Wellington Zoo. While specific reference ranges for rowi are not available for haematology parameters, when we extrapolated from other kiwi, all the birds at The Nest Te Kōhanga were showing a marked leukocytosis, with an absolute heterophilia, lymphocytosis and monocytosis.

Table 1
Faecal egg counts (eggs per gram of faeces) from rowi (*Apteryx rowi*) 1–3 days after admission to wildlife hospitals at Wildbase, Massey University and The Nest Te Kōhanga, Wellington Zoo. There are significant differences between the two hospitals for the three types of parasite ova identified. The results are presented as means (±one standard error).

	Wildbase	The Nest Te Kōhanga
Coccidia	56,850 (±22,122)	1250 (±366)
Capillaria	657 (±620)	19 (±13)
Strongyles	6 (±3)	56 (±33)

There was a significant difference ($F = 5733.96$, $df = 11,3$, $p < 0.001$) between the plasma biochemical analysis of birds kept in the two wildlife hospitals. There were significant differences in the plasma concentrations of phosphorus ($p < 0.001$), potassium ($p < 0.001$), sodium ($p = 0.18$), albumin ($p = 0.046$) and globulin ($p = 0.043$) (Table 3). However, none of the plasma biochemistry results on admission showed variation from reference ranges published for rowi and other kiwi (Morgan, 2008). It should be noted that the point-of-care analyser used has been criticised as being unreliable in its assessment of albumin and globulin (Greenacre et al., 2008) and the results should be interpreted with this in mind.

3.5. Radiography

Full body radiographs in two orthogonal views of 12 birds taken in the first week following admission to hospital showed no detectable abnormalities.

3.6. Enteric bacteriology

Cloacal swabs from 13 birds were negative on bacterial cultures for the presence of *Salmonella* spp and *Yersinia* spp.

3.7. Skin biopsies

Histological examination of the biopsies of the affected skin and vent margins showed mild to moderate epidermal hyperplasia with hyperkeratosis in all eight samples examined. In 7/8 biopsies, there were also marked perivascular to nodular aggregates of lymphocytes with fewer heterophils and eosinophils associated. In 5/8 biopsies, cross and tangential sections of nematode larvae were visible within the epidermis (Fig. 2). In several sections, there were intra-epidermal pustules comprised of small numbers of heterophils,

Table 2
Haematology from rowi (*Apteryx rowi*) 1–3 days after admission to wildlife hospitals at Wildbase, Massey University and The Nest, Wellington Zoo. Significant differences between birds kept in the different hospitals are highlighted in grey. (s.e. = one standard error). No eosinophils or basophils were detected in peripheral blood smears. No reference range is available for rowi. The reference range for brown kiwi (*Apteryx mantelli*) is from Morgan (2008).

	Wildbase		The Nest		Brown kiwi	GLM stats		
	Mean	(±s.e.)	Mean	(±s.e.)	Ref. range	F	df	p
Packed cell volume %	40.14	(1.62)	39.38	1.88	38–54	0.027	1	0.872
Total plasma solids (g/L)	40.00	2.89	43.83	2.88		0.872	1	0.370
WBCC ($\times 10^9$ cells/L)	20.60	1.51	67.40	7.00	8.7–14.5	42.980	1	0.000
WBCC corrected ($\times 10^9$ cells/L)	17.24	1.50	49.83	7.39		21.458	1	0.001
Heterophils ($\times 10^9$ cells/L)	9.04	0.91	25.03	4.44	4.0–8.2	25.825	1	0.000
Lymphocytes ($\times 10^9$ cells/L)	7.98	1.01	22.14	6.00	2.5–5.9	3.471	1	0.089
Monocytes ($\times 10^9$ cells/L)	0.22	0.07	2.46	1.00	0.1–0.5	8.229	1	0.015

Table 3
Plasma biochemistry from rowi (*Apteryx rowi*) 1–3 days after admission to wildlife hospitals at Wildbase, Massey University and The Nest, Wellington Zoo. Significant differences between birds kept in the different the hospitals are highlighted in grey. (s.e. = one standard error). The reference range for rowi (where available) is from Morgan (2008).

	Wildbase		The Nest		Rowi	GLM stats		
	Mean	(±s.e.)	Mean	(±s.e.)	Reference range	F	df	Sig.
AST ($U{\cdot}L^{-1}$)	156.71	16.48	177.75	36.89	132–276	0.245	1	0.629
CK ($U{\cdot}L^{-1}$)	804.29	75.91	1507.13	753.78	446–1071	0.748	1	0.403
Bile acids (μmol/L)	<35		<35		<35			
Uric acid (μmol/L)	125.14	31.66	92.88	12.30	343–610	0.999	1	0.336
Glucose ($mmol{\cdot}L^{-1}$)	7.97	0.26	7.68	0.26		0.626	1	0.443
Ca ($mmol{\cdot}L^{-1}$)	2.58	0.03	2.52	0.06	2.45–2.54	0.866	1	0.369
P ($mmol{\cdot}L^{-1}$)	1.67	0.05	2.03	0.06	1.80–2.23	22.081	1	0.000
Total protein (g/L)	44.57	2.07	48.25	2.20	47–57	1.456	1	0.249
Albumin (g/L)	26.14	0.91	23.38	0.86		4.846	1	0.046
Globulins (g/L)	18.57	1.41	24.75	2.25		5.045	1	0.043
K ($mmol{\cdot}L^{-1}$)	3.34	0.17	4.21	0.11		19.463	1	0.001
Na ($mmol{\cdot}L^{-1}$)	145.43	0.57	142.00	1.07	145–149	7.341	1	0.018

eosinophils, multinucleated giant cells and small amounts of granular necrotic debris.

Microbial cultures from six of the biopsies were carried out through a commercial veterinary diagnostic laboratory. In the three birds from Wildbase that had cultures from the skin biopsies, aerobic bacterial cultures identified coagulase negative *Staphylococcus aureus, Enterococcus* sp., and *Corynebacterium* sp. in all three birds. Anaerobic cultures of the skin biopsies grew low numbers of *Clostridium perfringens*. Fungal cultures were negative.

Microbial cultures from the skin biopsies from the three birds from The Nest Te Kōhanga grew a greater diversity of microorganisms. Aerobic bacterial cultures identified coagulase negative *S. aureus, Enterococcus* sp., *Corynebacterium* sp., *Escherichia coli, Klebsiella* sp., and *Proteus mirabilis*. Fungal cultures were positive for *Aspergillus fumigatus* in all three biopsies and *Fusarium solani* complex in one biopsy.

Three skin biopsies were dissolved overnight in pepsin/HCl and the digested material examined with a dissecting microscope for the presence of larvae; however, the skin failed to digest completely, possibly because it had been fixed in 70% alcohol and no nematode larvae were able to be identified.

Three mildly affected rowi that had not been previously sampled had skin biopsies four days after anthelmintic treatment as they showed increased pruritis and erythema of the ventral abdominal skin. Histological examination of these biopsies showed only mild epidermal hyperplasia and hyperkeratosis, with mild lymphocytic and eosinophilic dermatitis.

3.8. Molecular diagnostics

Skin scrapings of the lesions from 10 birds were negative by PCR testing for the presence of herpesvirus, poxvirus and Chlamydia.

Successful amplification of an appropriately sized PCR product (approx. 320 bp) was produced from all five rowi tissue samples in which larval nematodes had been identified histologically (Fig. 3). The sequences showed the highest similarity to members of the *Trichostrongylus* sp., specifically *T. axei* ($3e^{-130}$, KC998727) and *T. retortaeformis* ($5e^{-118}$, KC521412) with e-values of $<e^{-100}$. Further support of this result is demonstrated in the phylogenetic analysis (Fig. 4). These results demonstrate the association of the rowi sample with the *Trichostrongylus* genus.

Following the histological diagnosis of cutaneous larval migrans, birds were treated with anthelmintics. Birds at Wildbase Hospital were treated with moxidectin (Vetdectin, Fort Dodge, New Zealand) at 200 $\mu g{\cdot}kg^{-1}$ po once. As previously noted, three days after treatment all the seven rowi showed increased pruritis and erythema of the ventral abdominal skin. All the birds were then treated with meloxicam (Metacam, Boehringer Ingelheim, Manakau, New Zealand) at 0.5 $mg{\cdot}kg^{-1}$ po bid for 5 days. This resolved the pruritis and erythema within 48 hours. After discussions with Wildbase regarding the diagnosis of cutaneous larval migrans and the increased pruritis and erythema seen after treatment with moxidectin, the birds at The Nest Te Kōhanga were treated with fenbendazole (35 $mg{\cdot}kg^{-1}$ PO sid q4d) and meloxicam at 0.5 $mg{\cdot}kg^{-1}$ po bid for 5 days.

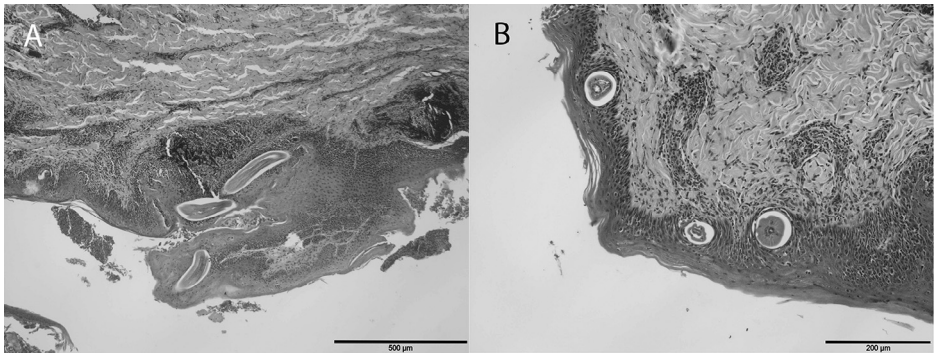

Fig. 2. Photomicrographs of skin biopsies taken from the ventral abdomen of rowi (*Apteryx rowi*) showing larval nematodes in tangential section (A) and cross section (B). Perivascular inflammation is evident in the dermis in both images.

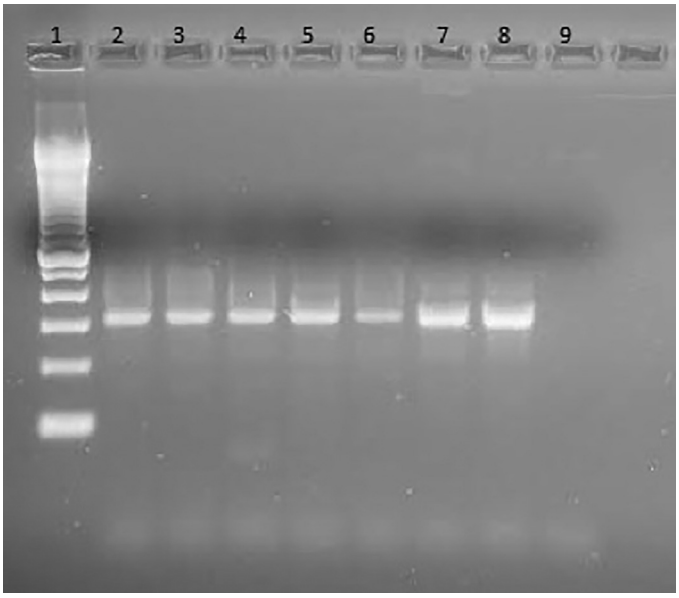

Fig. 3. PCR product from amplification of tissue samples from rowi with cutaneous larval migrans using nematode specific primers NC1 and NC2, separated and visualised on a 1% agarose gel. Lane 1 is the ladder, lanes 2–6 contain rowi samples, lane 7 is a control using LD plasmid, lane 8 is a positive control using a nematode (*Haemonchus contortus*), and lane 9 contains a negative water control.

3.9. Complications of hospitalisation

There were severe and lethal complications of the hospitalisation of these birds with 9 of the 15 birds dying during the investigation; one at Wildbase and eight at The Nest Te Kōhanga. This does not include the further death of the rowi chick with capture myopathy that had been making a good recovery from his initial presenting problems.

The first indication of complications was a leukocytosis with absolute heterophilia, lymphocytosis and monocytosis in all the birds at the Nest Te Kōhanga compared to the birds at Wildbase (Table 2). The first rowi that died was a bird that had been hospitalised earlier and was recovering from capture myopathy. This bird's death occurred after 32 days of its hospitalisation, but only two days after the arrival of the 14 rowi from the island. The arrival of the seven new birds coincided with all the birds (nine in total) at the Nest Te Kōhanga being shifted onto a bark-chip substrate. Once the post mortem and histology was performed it was identified that the bark chip was a possible source of fungal spores and the cause of complications. All the remaining birds, at the Nest Te Kōhanga, were removed from the bark substrate and placed on rubber matting. The post mortem findings from this and subsequent birds to die are presented in Table 4. All the birds that died at the Nest Te Kōhanga had evidence of severe pneumonia and pulmonary compromise that was confirmed by fungal cultures of lung tissue from deceased birds to be due to infection with *A. fumigatus*. The clinical presentation and post mortem findings are most consistent with exposure to overwhelming numbers of fungal spores. The most likely source of the fungal spores was the bark chip used as a substrate in the outdoor enclosure at the Nest Te Kōhanga. Further evidence for this was growth of *A. fumigatus* in fungal cultures of skin biopsies from the rowi at the Nest Te Kōhanga with no concurrent histological evidence of fungal infection. This suggests superficial external contamination of the skin. The skin biopsies of birds from Wildbase showed no growth of *Aspergillus* sp.

Birds at the Nest Te Kōhanga were treated for aspergillosis using 10 $mg{\cdot}kg^{-1}$ itraconazole orally twice daily and were nebulised with amphotericin B in sterile water. The initial nebulised dose of amphotericin B was 0.5 mg in 5 mL of sterile water, nebulised over 15–20 minutes bid. Three days later the nebulised dose was increased to 0.5 mg amphotericin B per mL of sterile water, with 5 mL total being nebulised over 15–20 minutes bid. Culture and sensitivity testing was performed on lung tissue from one deceased bird to determine if the infection was sensitive to the antifungal protocol being used. Results of this indicated that itraconazole (MIC: 0.125 mg/L), voriconazole (MIC: 0.125 mg/L) and amphotericin B (MIC: 0.5 mg/

Table 4
Summary of post mortem findings and causes of death of rowi chicks (*Apteryx rowi*) that died of complications associated with hospitalisation during an investigation of vent dermatitis. The first bird to die was a rowi chick that had been previously hospitalised for an unrelated traumatic leg injury and capture myopathy and is highlighted in italics in the table.

Date of death	Histological diagnosis	Cause of death
11/09/13	*1. Severe miliary mycotic pneumonia;* *2. Enteritis and typhlocolitis*	*Aspergillosis*
13/09/13	1. Severe miliary mycotic pneumonia; 2. Enteritis and typhlocolitis; 3. Verminous dermatitis	Aspergillosis
14/09/13	Severe miliary mycotic pneumonia	Aspergillosis
18/09/13	Severe miliary mycotic pneumonia	Aspergillosis
22/09/13	1. Acute renal tubular necrosis and heterophilic nephritis; 2. Renal, visceral and articular gout; 3. Verminous dermatitis; 4. Avascular necrosis and vasculitis of leg	Renal failure
25/09/13	1. Severe multifocal mycotic and foreign body pneumonia; 2. Pulmonary parabronchial smooth muscle hypertrophy; 3. Hepatic larval migrans	Aspergillosis
25/09/13	1. Severe multifocal mycotic pneumonia 2. Subacute myocardial necrosis 3. Pulmonary parabronchial smooth muscle hypertrophy	Aspergillosis
27/09/13	1. Severe multifocal mycotic pneumonia; 2. Pulmonary parabronchial smooth muscle hypertrophy	Aspergillosis
28/09/13	1. Pulmonary parabronchial smooth muscle hypertrophy; 2. Focal myocardial necrosis	Respiratory compromise
8/10/13	1. Cardiac fibrinous arteritis; 2. Pulmonary parabronchial smooth muscle hypertrophy	Respiratory compromise

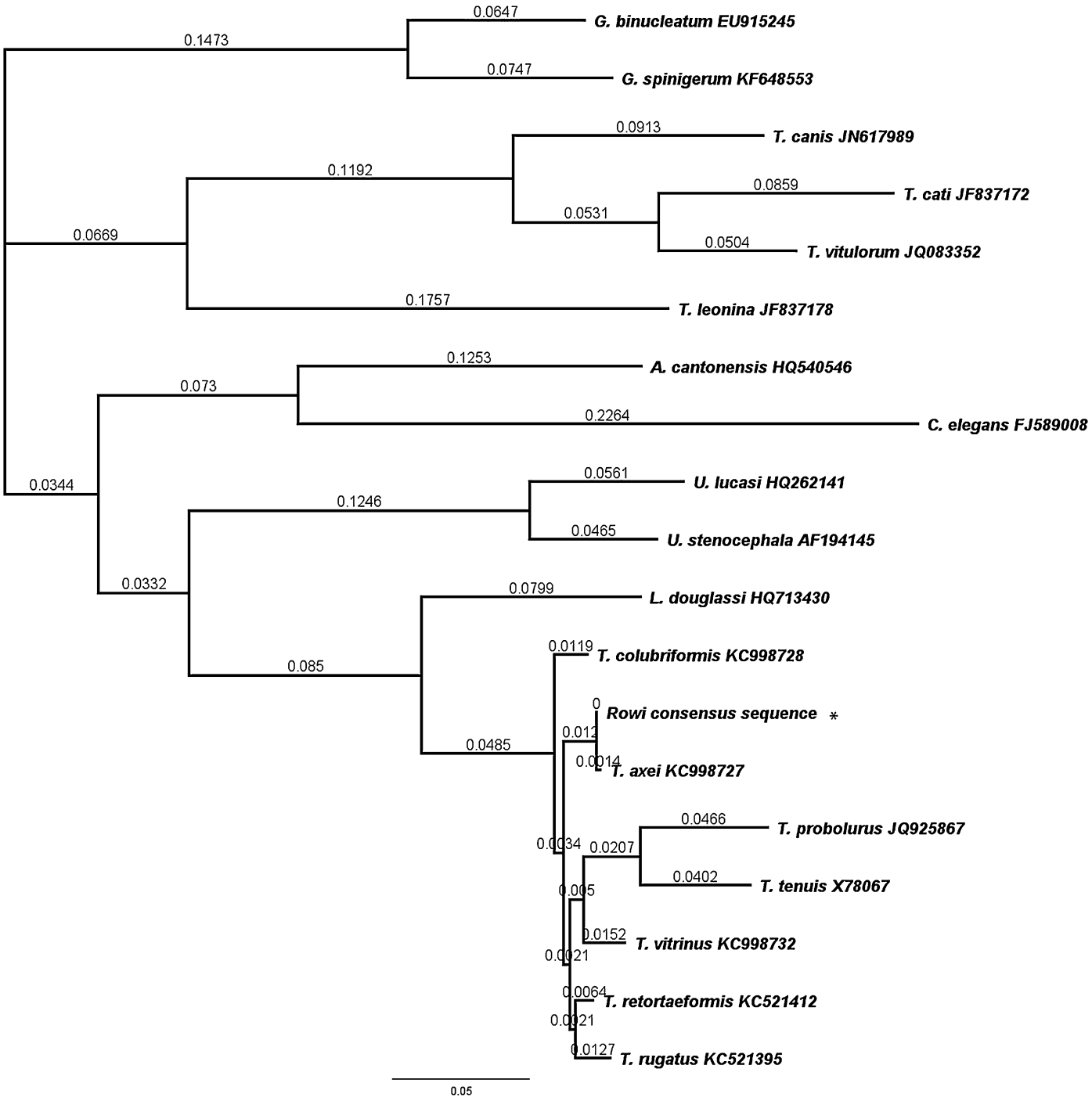

Fig. 4. Phylogenetic tree comparing 18 nematode species with published ITS 2 regions to the material extracted from the rowi (*Apteryx rowi*) skin samples (rowi consensus sample KM434192). We included *Caenorhabditis elegans* (FJ589008) as an outlier and model system. Other nematode species included; *Angiostrongylus cantonensis* (HQ540546), *Gnathostoma binucleatum* (EU915245), *G. spinigerum* (KF648553), *Libyostrongylus douglassi* (HQ713430), *Toxocara canis* (JN617989), *T. cati* (JF837172), *T. leonina* (JF837178), *T. vitulorum* (JQ083352), *Trichostrongylus axei* (KC998727), *T. colubriformis* (KC998728), *T. probolurus* (JQ925867), *T. retortaeformis* (KC521412), *T. rugatus* (KC521395), *T. tenuis* (X78067), *T. vitrinus* (KC998732), *Uncinaria lucasi* (HQ262141) and *U. stenocephala* (AF194145).

L) were effective against the strain of Aspergillus sp. cultured, but that the strain was resistant to fluconazole (MIC: 256 mg/L).

Despite this treatment, all birds at the Nest Te Kōhanga died with the subsequent deaths occurring at a range of 3–59 days of hospitalisation (median = 15 days). Later deaths showed histological evidence of increasing respiratory compromise despite treatment as evidenced by partial collapse of parabronchi, adjacent atria and to a certain extent, the adjacent air-capillaries, while parabronchial smooth muscle hypertrophy was common. Parabronchial, atrial and air-capillary epithelium was hyperplastic (cuboidal) while air-capillaries contained small amounts of eosinophilic fluid. In peripheral lung fields, parabronchial and adjacent air-capillaries were over-inflated.

A single bird died of renal failure at Wildbase, but showed no evidence of aspergillosis and this death is thought not to be linked to the deaths at the Nest Te Kōhanga, although a cause of the renal

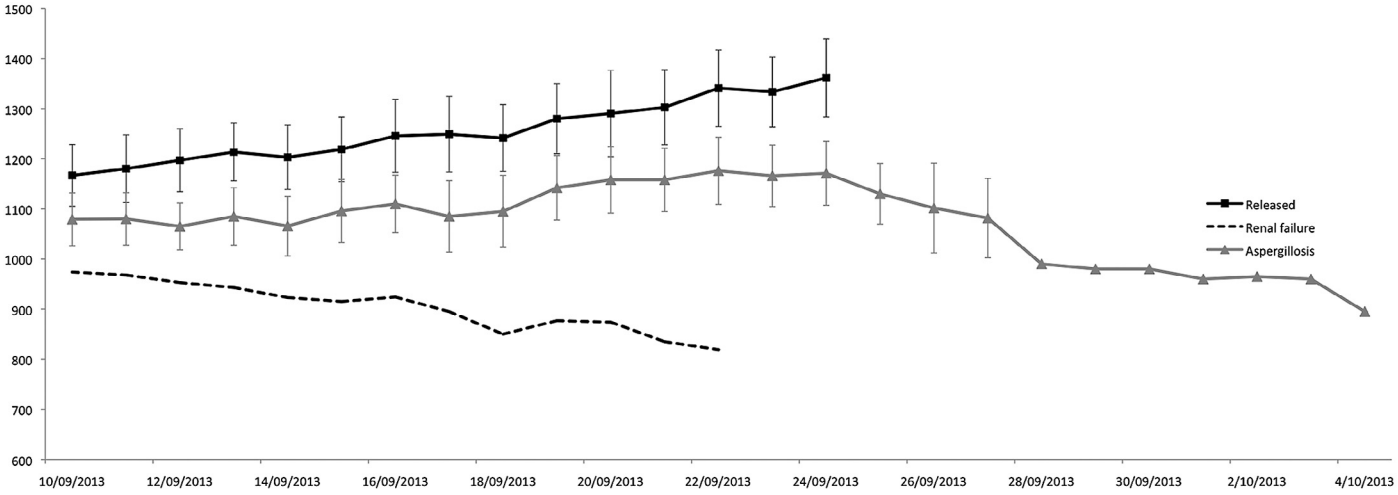

Fig. 5. Body weight change of rowi (*Apteryx rowi*) hospitalised for investigation of ventral dermatitis showing mean weights for birds that were released back to the crèche island and birds that died of aspergillosis. Error bars represent ± one standard error. The bodyweight over time of a single bird that died of renal failure is also presented.

failure was not established. This bird showed inappetance and weight loss over its period of hospitalisation despite hand-feeding (Fig. 5). The bird had been blood sampled once weekly during its hospitalisation and showed no clinicopathological abnormalities in haematology and plasma biochemistry until a blood sample taken the day before it died which showed hyperuricaemia (2975 Umol·L^{-1}), hyperkalaemia (6.2 mmol·L^{-1}) and a mild elevation of plasma creatine kinase (2745 U·L^{-1}). The post mortem (Table 3) showed an unusual presentation of visceral and articular gout, but no cause for the renal failure could be determined. There was an avascular necrosis of the intravenous fluid site which suggests a thrombophlebitis secondary to catheterisation but this would not have induced renal failure.

4. Discussion

4.1. Cutaneous larval migrans

Cutaneous larval migrans has not previously been reported in birds, although it is commonly seen in humans (Chaudhry and Longworth, 1989; Davies et al., 1993; Hochedez and Caumes, 2007) and in mammals such as New Zealand sea lions (*Phocarctos hookeri*) (Acevedo-Whitehouse et al., 2009) associated with hookworm species. A rarer form of cutaneous larval migrans in people known as gnathostomiasis is associated with ichthyophagous birds (Rusnak and Lucey, 1993; Kraivichian et al., 2004; Herman and Chiodini, 2009). *Gnathostoma* species are a potential cause of the cutaneous larval migrans seen in the rowi, as little penguins (*Eudyptula minor*) and other fish-eating seabirds such as sooty shearwater (*Puffinus griseus*) and fluttering shearwater (*P. gavia*) are known to use burrows on the crèche island. If hookworm larvae are involved, this infection may have originated from New Zealand fur seals (*Arctocephalus forsteri*) that occasionally use the island as a haul out (T. Makan, pers. comm.).

The molecular identification of the nematode larvae in the skin biopsies as *Trichostrongylus* species was unexpected and should be viewed with some caution, especially as we were unable to recover any whole nematode larvae for morphological assessment. This genus of nematodes has been recorded in a number of hosts including birds, humans, lagomorphs and ruminants (Drudge et al., 1955; Callinan, 1978). *Trichostrongylus* species have been recorded in a number of avian hosts, particularly ground dwelling birds like grouse (*Lagopus lagopus scoticus*) (Saunders et al., 1999), partridges (Purdy et al., 2012), poultry (Sherwin et al., 2013), snow geese (Shutler et al., 2012) and capercaillie (Millán et al., 2008). There are no previous records of *Trichostrongylus* species being involved in cutaneous larval migrans in any species (Tompkins, 2008). However, the infective third stage *Trichostrongylus* larvae show active migration in the environment to improve their chances of being consumed by hosts (Saunders et al., 1999; Tompkins, 2008).

The technique used in the molecular identification of this parasite involved the examination of the second internal transcribed spacer (ITS-2) of the ribosomal DNA. Congeneric species of parasitic helminths usually differ in the sequence of the ITS-2 region of their rDNA (Hoste et al., 1995). It has been shown that rDNA genes and their associated spacer regions are suitable targets for developing diagnostic markers for species identification of *Trichostrongylus* (Hoste et al., 1995). Hoste et al. (1995) have also shown that by using a PCR-linked RFLP method, the ITS-2 region of *H. contortus, Teladorsagia circumcincta, Cooperia oncophora, Trichostrongylus colubriformis, T. axei* and *T. vitrines* can be distinguished from each other, which implies that they differ in their nucleotide sequence and that this amplicon is thus useful for species identification (Hoste et al., 1995; Bott et al., 2009). Further, there were consistent interspecific differences between the five nematode species examined; however, the level of interspecific differences in nucleotide sequence was low (Hoste et al., 1995). Given this lack of interspecific variation, we cannot confidently exclude other members of the *Trichostrongylus* species as the cause of the cutaneous larval migrans in the rowi.

4.2. Environmental factors contributing to infection

The conservation management and the unusual communal roosting behaviour of young rowi are likely to increase the risks of transmission of parasites between birds. The roost boxes on the crèche island had also not been moved or cleaned for approximately five years further contributing to the focal environmental loading of parasite eggs and larvae. This was evident not just by the unusual presentation of cutaneous larval migrans but also by the clinical diagnosis of coccidiosis and gastro-intestinal helminthiasis in most birds from the island. Subsequent to this investigation, changes to management of the birds on the island included replacing the old roost boxes and shifting them to new sites. It is strongly recommended that the roost boxes be cleaned or replaced regularly,

preferably annually as a minimum. The site of the roost boxes should also be moved regularly.

The use of the predator free crèche island has undoubtedly been of tremendous benefit to the conservation of the rowi. However, the island eco-system is markedly different from the mainland habitat of these birds and we suggest that the cutaneous larval migrans seen in the rowi is most likely due to cross-species transmission from either seabirds or, less likely, marine mammals that visit the island. While excluding seabirds from the burrows would be ideal to reduce the possibility of cross-species infections, the feasibility of this is doubtful.

4.3. Treatment and management of cutaneous larval migrans

In humans and mammals, cutaneous larval migrans is usually a self-limiting condition but can occasionally result in chronic disease. Recommendations for treatment of cutaneous larval migrans in people include the use of anthelmintics such as albendazole, mebendazole, levamisole, or pyrantel pamoate (Van Den Enden, 2009). The treatment of the rowi with moxidectin alone resulted in a temporary worsening of dermal inflammation and pruritis, potentially associated with the death of larval nematodes. Treatment with meloxicam, a non-steroidal anti-inflammatory agent, rapidly ameliorated the pruritis and inflammation. If future cases of this ventral dermatitis are seen in the young rowi on the island, then we recommend *in situ* treatment with oral moxidectin or ivermectin and a single oral dose of meloxicam. For birds in captivity, anthelmintic treatment with a benzimidazole, such as fenbendazole, given orally over 3–5 days may be more effective and result in less pruritis and inflammation.

Cases of larval migrans have been recorded in young brown kiwi (*Apteryx mantelli*) in other organs, such as the liver, lung and brain (Gartrell, 2014). Work is currently in progress to identify the species of nematodes and their primary hosts, although it is likely that introduced species of mammals and birds are the most likely primary hosts. The death of other native birds has been due to cross-species transmission of nematodes. For example, *Porrocaecum* species of nematodes have caused intestinal rupture and peritonitis in saddlebacks (*Philesturnus carunculatus*) (Alley et al., 2007). The normal host species of this nematode are blackbirds (*Turdus merula*) and starlings (*Sturnus vulgaris*), illustrating the indirect impacts that invasive species can have on native birds. Identifying the species and host of the nematodes involved in this syndrome of cutaneous larval migrans will be essential in preventing its occurrence in the future.

The decision to remove the rowi from the island for the diagnostic investigation and treatment was made between wildlife veterinarians and conservation managers based on the perceived similarity of the vent dermatitis to a similar problem encountered in kakapo (*Strigops habroptilus*). In kakapo there is a syndrome of ulcerative vent dermatitis of unknown aetiology that has required hospitalisation, debridement and intensive medical treatment to resolve (Jakob-Hoff and Gartrell, 2012). Only one of the 15 birds hospitalised had a vent dermatitis that was severe enough to require this degree of treatment.

4.4. Aspergillosis

Aspergillosis is a common complication in captive and hospitalised birds in a variety of species (Beernaert et al., 2010). Infection in birds is generally thought to be due to either immunocompromise or exposure to high spore levels that overwhelm the host defences. There have been previous mortalities of captive kiwi in nocturnal houses that have been shown to be due to high levels of *Aspergillus* contamination of substrate, both leaf litter and bark chip (Glare et al., 2014). A recent survey of the substrate of nocturnal houses for captive kiwi showed all substrates carry some levels of *Aspergillus* species in the substrate, and that no commercially available and rapid method exists to screen substrate for the levels of fungal contamination (Glare et al., 2014). There is a recommendation to avoid the use of eucalyptus mulch and bark chip in the Kiwi Captive Husbandry manual (Fraser and Johnson, 2011), but this is based on studies that report this being a source of *Cryptococcus* rather than *Aspergillus* species.

In the case of the deaths of the rowi from aspergillosis, it is likely there was an overwhelming exposure to fungal spores originating from the bark chip used as substrate. It is also possible that there were other potential contributing factors in these cases including the relatively low genetic diversity of this critically endangered species, which may have effects on immune response (Shepherd and Lambert, 2008). The stress of hospitalisation may have also played a role in the severity of the disease seen in the birds. Aspergillosis is a notoriously difficult disease to treat in birds (Beernaert et al., 2010). The treatment protocols used by The Nest Te Kōhanga were current best practice and it is both surprising and disappointing that no birds responded to treatment. This is most likely due to the severity of the initial exposure. Later deaths showed reduced fungal elements in sections of lesions and the cause of death was assessed by the pathologist as being more likely due to respiratory compromise and complications associated with the host response to the infection, rather than primarily due to the pathogen itself.

4.5. Conclusions

In summary, this investigation has identified cutaneous larval migrans, possibly from a species of *Trichostrongylus* as a cause of ventral dermatitis and occasional ulcerative vent dermatitis in rowi. The predisposing factors that led to this disease are suspected to be the novel exposure of the rowi to parasites from seabirds or marine mammals due to the island crèche and the limited management of roost boxes on the crèche island. This is the first instance of cutaneous larval migrans to be recorded in birds. Severe and fatal complications of the investigation resulted in the death of eight birds of aspergillosis and pulmonary complications associated with the use of bark as a substrate. Another bird died of renal failure during the period of hospitalisation despite oral and then intravenous fluid therapy. The initiating cause of the renal failure was not determined. This set of complications has the potential to undermine the working relationship between wildlife veterinarians and conservation managers. This disease highlights that intensive conservation management can result in increased opportunities for novel routes of cross-species pathogen transmission.

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Conflict of interest

The authors declared that there is no conflict of interest.

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