

Observations on Avipoxvirus Infections in Brown Kiwi, *Apteryx mantelli*, in a Captive-Rearing Facility

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Abstract

Avipoxvirus infections, already known to occur in brown kiwi, *Apteryx mantelli*, were observed and confirmed in 2 juvenile kiwi (1 bird in 2013, the other in 2015) within the Kiwi Encounter captive-rearing facility. This paper includes a sequence of photographs tracking the physiological changes during treatment. An earlier infection occurred, in 2012, in another young kiwi being raised in the facility which was only subsequently confirmed via Polymerase Chain Reaction (PCR) testing.

Kiwi Encounter, at Rainbow Springs in Rotorua (38° 08' S, 176° 14' E) is the largest facility in New Zealand for the artificial incubation, hatching and rearing of brown kiwi, *Apteryx mantelli*. It operates as part of the "Operation Nest Egg" kiwi recovery programme (Colbourne and others, 2005) and hatches about 100 brown kiwi per year.

In both cases, the infections first appeared on the distal portion of the right side of the upper mandible (i.e. upper part of the bill). The lesions increased rapidly in size until treatment began. The first case was transported to Wildbase in Palmerston North and treated twice daily for a month with antibiotics (amoxicillin/clavulanic acid, at 125 mg/kg) and topical iodine solution before being returned to Kiwi Encounter with only a small keratin deficiency remaining. The bird was released to the wild, at a bodyweight of 1.2 kg, 90 days after the infection had first been seen. The second bird was kept at Kiwi Encounter, in isolation, and given the same basic treatment as the first case and was also able to be released to the wild. Changes to management protocols of the affected birds included avoidance of aggressive debridement and leaving the lesion unsealed.

The avipoxvirus seems to be widespread in the environment, but the incidence of infections has been low. The infections seen at Kiwi Encounter are likely to have followed trauma to the juvenile birds' bills.

Key words: *Apteryx mantelli*, avian pox, avipoxvirus, bill lesions, brown kiwi, captive rearing.

Introduction

Avipoxvirus is a common cause of disease in birds worldwide (Van Riper and Forrester, 2007). It is an encapsulated virus so it survives well in the environment (Tripathy and Reed, 2008). Transmission can be by direct contact, contaminated environment or via insect vectors (Gartrell and others, 2003; Tripathy and Reed, 2008). The cutaneous form of the infection can only penetrate the body through a break in the skin or via mucous membranes, and typically shows on the non-feathered regions of the bird's body as proliferating nodules (Dowding and O'Connor, 2013).

Avian pox is known to occur in a variety of endemic and native New Zealand birds and at least two distinct sub-clades have been identified in New Zealand (Ha and others, 2011; (Gartrell, pers. comms. 2015). Pox lesions in brown kiwi, *Apteryx mantelli*, were first described by Ha and others (2013) who reported avipoxvirus nodules on 2 juvenile wild brown kiwi on Ponui Island (36° 52' S, 175° 11' E, Figure 1), on the leg of one in 2008 and the bill of the other in 2011. The presence of avipoxvirus was confirmed by Polymerase Chain Reaction (PCR) testing, with the bird with lesions on the leg having carried the A3 strain of avipoxvirus, while the other bird carried the B1 strain of avipoxvirus at the base of the bill (Ha and others, 2013).

Kiwi Encounter, at Rainbow Springs in Rotorua (38° 08' S, 176° 14' E, Figure 1) is the largest facility in New Zealand for the artificial incubation, hatching and rearing of brown kiwi. It operates as part of the "Operation Nest Egg" kiwi recovery programme (Colbourne and others, 2005) and follows a standard procedure in which kiwi eggs collected from the wild, incubated and the hatched chick is reared to a "stoat-proof" bodyweight of 1 kg before being released back to the area the egg was lifted from, thereby increasing survival rates of kiwi from 5% to 65% (McLennan and others, 1996). Whilst at Kiwi Encounter, kiwi chicks are fed an artificial diet (Potter and others, 2010) and are kept indoors in purpose-built brooders until they re-attain hatch weight (at approximately 3 weeks old), after which they are transferred to an outdoor enclosure. Juvenile kiwi are either housed on their own or in a small group of comparable age and size. As per protocol, food consumption is measured daily as an indication of bird health, and the birds are weighed weekly as per the stool-sampling regime and are treated for internal parasites as required (Bassett, 2012). Juvenile kiwi stay on-site for an average of 3 months, and are released into the wild at over 1 kg bodyweight, after passing quarantine tests. Between 2012-2017, the total numbers of birds released as part of Operation Nest Egg from Rainbow Springs each year were 123, 89, 92, 102 and 130 respectively.

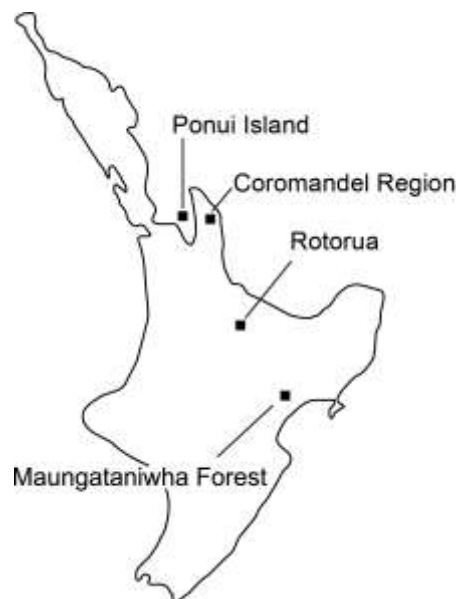


Figure 1. Diagram showing locations mentioned in the text.

The present report describes avipoxvirus infections (in 2013 and 2015) in two juvenile kiwi hatched at Kiwi Encounter on the distal portion of the right side of the upper mandible. The term "upper mandible" is used to describe the upper part of a bird's bill, in accord with common ornithological usage (Heather and Robertson, 2015). The bill of the kiwi is distinctive in having the left and right nostrils close to the distal (free) end of the bill. Many of the nerve endings in the region of the nostrils (and also more proximally along the bill) are mechanoreceptors (Cunningham and others, 2007; Cunningham and others, 2013). The present report also discusses two historical (2012) cases of kiwi which had swellings on their bills – one of which was subsequently confirmed by PCR testing to be avipoxvirus.

Clinical Histories, Signs and Treatments

Case 1

The subject was a 3 month old female brown kiwi chick, *Apteryx mantelli*, being raised after a normal incubation and hatching, with no previous health concerns. The lesion on the chick's bill was first noted on 09 May 2013 - cited as Day 1 on Table 1. The bird was 87 days post-hatch, weighed 763 g and had been in an small-group outdoor enclosure for 54 days.

When first noted the lesion appeared as 8 x 2 mm area of raised soft tissue, midway along the right side of the upper mandible. One week later the affected area was approximately four times its initial size (Figure 2). A biopsy sample, taken the next day, was sent for histological processing and revealed a severe ulcerative dermatitis with possible intraepithelial pox-virus inclusions. A separate Polymerase Chain Reaction (PCR) test showed the presence of the B1 strain of avipoxvirus. Meanwhile, the bird was isolated, and treated twice daily with antibiotics ("Clavulox tablets", Pfizer (NZ) Ltd, Auckland – a combination of clavulanic acid and amoxicillin) given orally at 125 mg/kg (Morgan, 2008). The lesion did not affect the birds ability to eat - standard consumption amounts for this bird continued to be recorded.

On 26 May 2013, the bird was transferred to Wildbase, at Massey University in Palmerston North and continued the twice daily treatment with clavulanic acid and amoxicillin ("Amoxyclav", GlaxoSmithKline Ltd, Auckland) at 125 mg/kg, together with topical iodine and oral fluids. When the bird arrived back in Rotorua on 24 June the bill was almost back to normal, the lesion having regressed to leave only a small keratin deficit (Figure 3) beneath which the tissues were faintly pinkish in colour, following the sloughing of scar tissue. At this stage the bird was believed to be no longer infectious and it was returned from indoor isolation to another outdoor enclosure while the bill repair continued.

Within 6 weeks the bill appeared normal again, apart from the static small keratin deficit. As hosts surviving avipoxvirus infection usually acquire lifelong immunity (Wagner and other, 2008) and due to the prevalence of avipoxvirus in the wild, the chick was deemed suitable for release (McInnes pers. comms. 2013). The bird was in good health and released into the Maungataniwha forest on 07 August 2013, weighing 1.2 kg, close to the location from which it was located as an egg.

Significant features of Case 1 are summarised in Table 1 and Figures 2 and 3.

Day*	Date	Event
1	09.05.13	First noted a raised area of soft tissue on right side of upper mandible, size 8 x 2 mm. Consulted local vet and advised to reassess in 1 week. The bird was 87 days old, weighed 763g and had been in the outdoor enclosure for 54 days.
8	16.05.13	Raised area now approximately 4 times larger than it was last week. Photograph taken (Figure 2). No corresponding issues on the underside of the bill or inside the mouth.
9	17.05.13	Taken to local veterinary clinic, for biopsy. Then transferred to indoor pen for isolation. Began antibiotic therapy (Clavulox 125mg/kg BID)
15	23.05.13	Histology report is consistent with avipoxvirus infection.
18	26.05.13	Bird transferred to Wildbase, Palmerston North, for continued treatment.
46	23.06.13	Bird arrived back at Kiwi Encounter for further rehabilitation in an outdoor enclosure and is no longer considered infectious.
78	25.07.13	Small keratin deficit in bill remains. Photograph taken (Figure 3).
91	07.08.13	Bird released into Maungataniwha Forest. Photograph taken (Figure 3).
* Day = the individual day within the overall period of observations, e.g., Day 1 (09 May 2013) was the day on which the lesion was first noticed. The date on which the bird became infected is not known; nor is the date (during treatment) on which the bird became free of infection.		

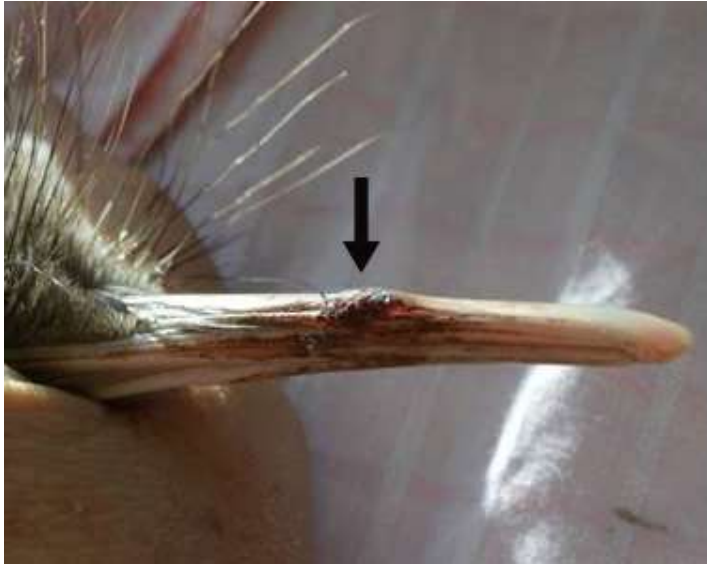


Figure 2. Case 1: Avian pox lesion (arrowed) midway along the right side of the upper mandible of a 3 month old female kiwi chick as seen on Day 8 (i.e. 16.05.13, the eighth day of observation, Table 1). The lesion had increased to approximately four times its size when first seen a week earlier. Photograph taken by the author.



Figure 3. Case 1: The day the bird is release back to the wild, a small keratin deficit remained. Left: Dorsal view of bill and Right: Dorsolateral view of bill on Day 91 (07/08/13). Photographs taken by the author.

Case 2

The subject was a male brown kiwi chick, *Apteryx mantelli*, again 3 months old and housed outside when it was first noted to have a small irregularity on the right side of its upper mandible. Relatively rapid changes in the lesion made us suspect a pox infection, and avipoxvirus was confirmed by histological testing and further identified by PCR testing which revealed the same B1 isolate as in Case 1.

In this case, we were advised to keep this bird isolated and treat it on-site, despite the lesion being close to the right nostril. The treatment consisted of twice daily oral antibiotic "Clavulox" tablets (Pfizer (NZ) Ltd, Auckland) at 125 mg/kg and topical application of iodine ("Betadine", Consumer Medicare, Auckland - containing providone and iodine). We were advised to discontinue the

Clavulox when the lesion developed a dry scab -- this occurred after 4 weeks of treatment. We were advised to continue treatment of the scabbed area with a topical iodine for a total of 8 weeks, until the dry keratin sloughed off to reveal smooth healed keratin underneath. At this point, the patient was considered non-infectious and was removed from isolation. The bird was released to the wild (in the Coromandel region), at a bodyweight of 1.2 kg, 4 months after the avipoxvirus infection was first noted.

Significant features of Case 2 are summarised in Table 2 and Figures 4 to 7.

Day*	Date	Event
1	19.05.15	First noted a small irregularity on right side of upper mandible, 8 x 2 mm in size. The bird was 84 days old, weighed 965g and had been in the outdoor enclosure for 52 days.
3	21.05.15	No change in diameter, but lesion more raised – decision to reassess at next scheduled health check. Photograph taken (Figure 4).
8	26.05.15	Lesion now more clearly delineated, veterinary advice sought. Photograph taken (Figure 4).
10	28.05.15	Small biopsy sample obtained. Began oral antibiotic treatment (Clavulox 125mg/kg BID) and topical iodine, plus a one-off meloxicam (“Metacam”, Zoetis, Auckland) injection given post-surgery for pain relief. Bird isolated inside and housed on non-slip matting (not soil). Routine stool sampling and dosing protocol was adhered to throughout the treatment. The bird continued to self-feed for the duration of treatment. Photograph taken (Figure 4).
12	30.05.15	Histology confirms avipoxvirus by the presence of intra-cytoplasmic inclusion bodies.
15	02.06.15	Lesion extending toward nostril. Photograph taken (Figure 5).
16	03.06.15	Re-examination by veterinarian of lesion in proximity to the right nostril – advised extra care required when applying topical iodine as it is an irritant if inhaled.
18	05.06.15	Lesion now 10 mm in length. Photograph taken (Figure 5).
21	08.06.15	Staining of lesion by iodine more obvious. Photograph taken (Figure 5).
30	17.06.15	No fresh swelling – still a large, drying lesion. Photograph taken (Figure 5).
37	24.06.14	Discontinued antibiotic treatment (total 4 week course) but continued with topical iodine.
39	26.06.15	Lesion seems to be drying. Photograph taken (Figure 5).
45	02.07.15	Keratin drying and lifting.
49	06.07.15	Central, active part of lesion now more clearly confined. Photograph taken (Figure 6) .
67	24.07.15	Discontinued use of topical iodine (end of 8 week treatment).
71	28.07.15	Re-examination by veterinarian, who was happy with bird's progress. No need to surgically debride. NB debridement of non-active scab if required – <i>never</i> debride active avipoxvirus.

77	03.08.15	Scab had sloughed naturally, leaving an apparently-healthy, pink coloured deficit in the bill. Photograph taken (Figure 6).
80	06.08.15	Soil added to brooder, to encourage bird's natural probing behaviour.
82	08.08.15	Natural probing behaviour pushes soil up under part of keratin. Photograph taken (Figure 6).
85	11.08.15	Keratin further discoloured and raised, as sloughing continued. Photograph taken (Figure 6).
92	18.08.15	Moved to outdoor enclosure.
96	22.08.15	The remainder of the dry keratin has sloughed off and signs of probing activity can be seen in enclosure. Photograph taken (Figure 6).
101	27.08.15	Now only a small deficit remained in keratin. Photograph taken (Figure 7).
108	03.09.15	Quarantine samples collected, for health checks prior to release of bird.
130	24.09.15	Bird released to the wild (in Coromandel region) at 1.2kg. Photograph taken (Figure 7).

* Day = the individual day within the overall period of observations, e.g. Day 1 (19 May 2015) was the day on which the lesion was first noticed. The date on which the bird became infected is not known; nor is the date (during treatment) on which the bird became free of infection.

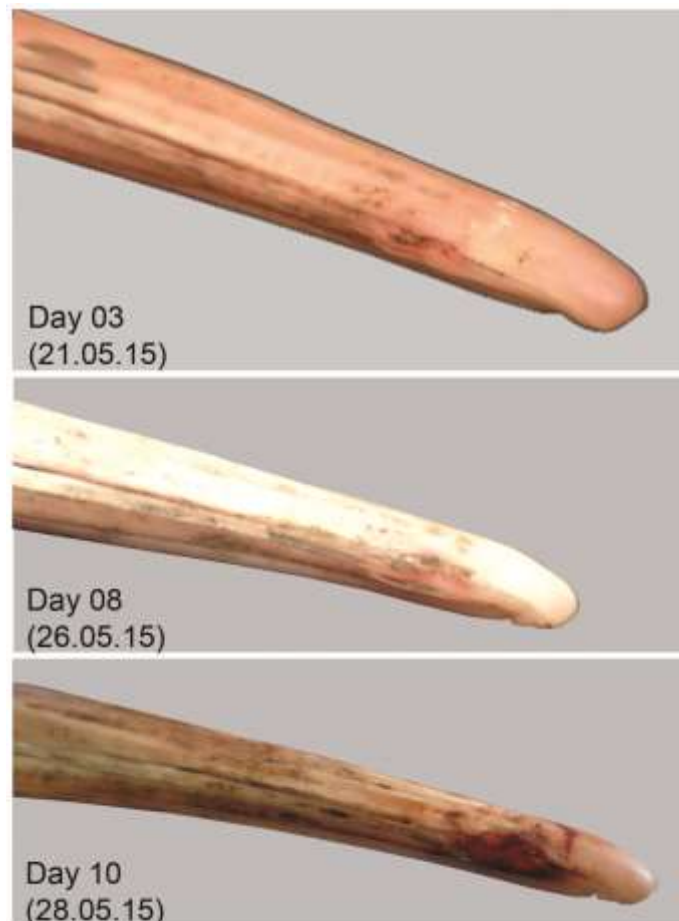


Figure 4. Case 2: Successive stages in early growth of the lesion on the upper mandible. For each stage, the day of observation and the date are stated - see Table 2. Photographs taken by the author.



Figure 5. Case 2: Successive changes in lesions during the first month of treatment. For each stage, the day of observation and the date are stated - see Table 2. Photographs taken by the author.

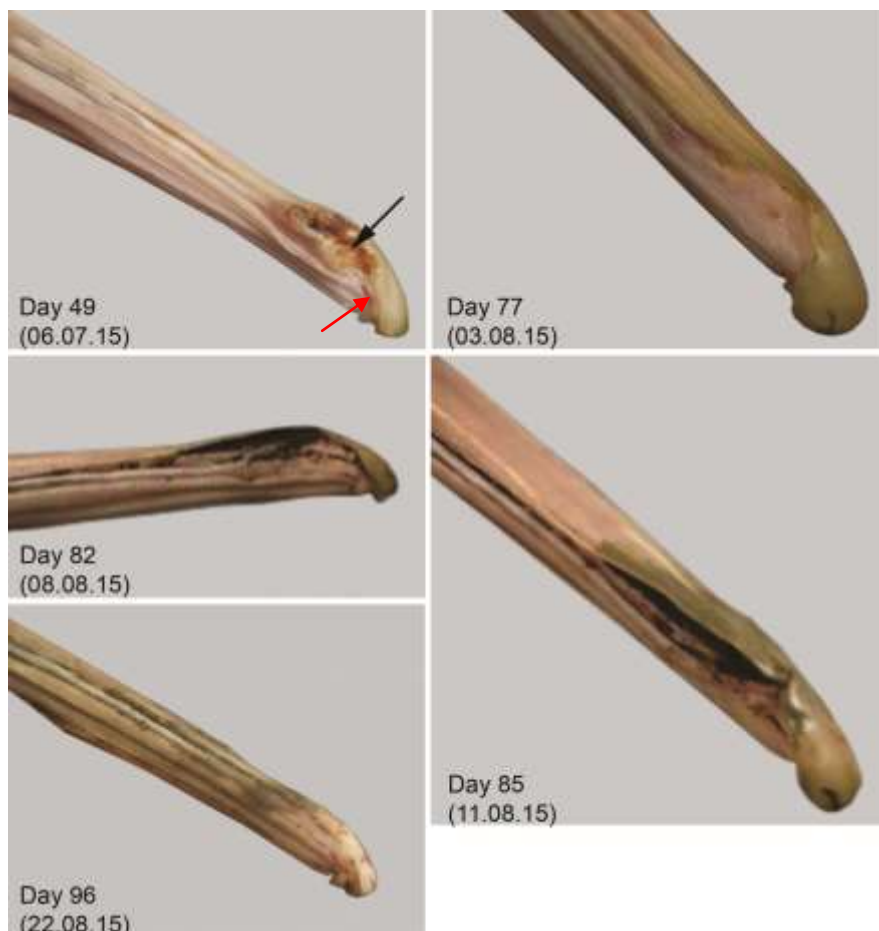


Figure 6. Case 2: Successive later stages in recovery. For each stage, the day of observation and the date are stated – see Table 2. The image at top left (Day 49) shows that the lesion (black arrow) is in the region of the right nostril (red arrow) but the airway is still patent. Photographs taken by the author.



Figure 7. Case 2: Above: Dorsolateral views of bill on Day 101 (27.08.15) when the bird was moved into an outdoor enclosure. Below: Day 130 (24.09.15) when the bird was being released into the wild. Photographs taken by the author.

At the time of completing the present report, no signs of avipoxvirus infection have been seen in any of the kiwi chicks hatched at Kiwi Encounter during the 2016-2017 season.

Reassessments of Bill Lesions in Two Earlier Kiwi

In 2012, Kiwi Encounter had had 2 juvenile brown kiwi with raised soft tissue on their bill. One of these birds, a 5 month old male, had exuberant formation of granulation tissue near the distal end of the bill (Figure 8). The other bird had a small swelling near the base of its bill (Figure 9).

Initial histological examinations of biopsy samples from both birds could not confirm that the lesions were due to avipoxvirus. The histology block from the 5 month old male kiwi was kept in storage and later sent for PCR testing. This testing revealed that the bird had the B1 strain of avipoxvirus, presented by the lesion in Figure 8. Unfortunately, the sample from the other bird had not been retained, however the lesion did not show the rapid changes exhibited in the other three cases. In addition, the swelling at the base of the bill shown in Figure 9. gradually reduced and was fully healed within a month, indicating that the lump was likely to have resulted from a traumatic injury rather than an avipoxvirus infection. Furthermore, the case occurred in March, whereas the other cases were all first noted in May.



Figure 8. Distal portion of right side of the upper mandible of a 5-month-old kiwi, showing presence of exuberant granulation tissue in the region of the right nostril. Photograph taken by the author.



Figure 9. Left side of the base of bill of a juvenile brown kiwi, showing diffuse enlargement (arrowed) which then steadily diminished within the next month and was thought likely to have resulted from mechanical trauma and not infected with avipoxvirus. Photograph taken by the author.

Changes to Management Protocols

As a result of the multiple avipoxvirus cases, Kiwi Encounter has improved its management protocols and techniques. Lesions suspected of being caused by avipoxvirus infection are no longer aggressively debrided, as this can aggravate an avipoxvirus infection (Gartrell, pers. comms. 2015). Instead, a small biopsy sample is taken for PCR testing. The biopsy site is no longer sealed, because sealing can trap any avipoxvirus present and it is better to encourage the infection to travel outwards (Gartrell, pers. comms. 2015). The aim is to minimise the spread of avipoxvirus by isolating infected individual birds, using biosecurity principles, disposing of all consumable used, and decontaminating items which cannot be disposed of, by immersing them for 24 hours in a 20 ml/L solution of disinfectant ("SteriGENE®", Ethical Agents, Auckland) active against bacteria, fungi, protozoa, spores and viruses, or an another such disinfectant solution.

Discussion

The present report describes and illustrates the progression and healing of avipoxvirus lesions on the bills of 3 juvenile brown kiwi, *Apteryx mantelli*. The course of an avipoxvirus infection can be highly variable, but can take several weeks to resolve and typically exhibits low mortality (Gartrell and others, 2003). This report confirms that with time and appropriate treatment the lesions can resolve completely, allowing the release of kiwi, *Apteryx spp*, with functional sensory pits (containing mechanoreceptors) and normal foraging ability (Cunningham and others, 2007; Cunningham and others, 2013). However, avipoxvirus infection can result in secondary bacterial or fungal infections which can complicate recovery (Tripathy and Reed, 2008). Cutaneous avipoxvirus infection by A1 and B1 isolates are believed to be the cause of on-going mortalities in shore plovers, *Thinornis novaeseelandiae*, populations (Ha and others, 2011; Dowding and O'Connor, 2013). Therefore, despite documented cases of spontaneous resolution of lesions in the wild (Ha and others, 2013), isolation of avipoxvirus-infected individuals and prophylactic treatment is advisable to reduce transmission to others in a population and prevent secondary infection in the affected individual, regardless of the additional cost of rearing them.

Avipoxvirus is a well-encapsulated virus which survives for months or years in the environment and is frequently present on fomites and dried scabs (Tripathy and Reed, 2008). The means of transmission of avipoxvirus to brown kiwi is yet to be determined. Cutaneous avipoxvirus is often

transmitted by biting insects or through breaks in the skin (Gartrell and others, 2003; Dowding and O'Connor, 2013). Avipoxvirus infection may also be determined largely by a bird's level of immunity, which correlates with observations that juvenile birds appear more susceptible to avipoxvirus infection than adults (Dowding and O'Connor, 2013).

The present report indicates that time of year could also be a factor, as all 3 confirmed cases were first observed in May (i.e. during late autumn/early winter conditions in New Zealand). Dowding and O'Connor (2013) correlate seasonal peaks in infection to higher numbers of biting insects. Avipoxvirus requires a break in the host's epithelium for infection to become established. As insects are unlikely to bite bill keratin, the primary route of avipoxvirus infection in a kiwi's bill is still uncertain, but is more likely to follow bill trauma or abrasion of the nasal epithelium (pers. comms. Gartrell, 2016).

During the development and resolution of the avipoxvirus lesions, a variety of morphological stages were exhibited and these are often obvious in appearance, which raises the question of why they have not been recorded in brown kiwi until recently. Whilst avipoxvirus is widespread in New Zealand, Ha and others (2011) report that the most prevalent avipoxvirus strain is a different sub-clade from the B1 isolate identified in brown kiwi; which may explain the low incidence of avipoxvirus observed in brown kiwi to date. A study of seroprevalence of avipoxvirus in wild kiwi populations would be useful to clarify the extent of exposure and immune response to the virus and so reveal its significance in kiwi conservation management.

Conclusions

1. Avipoxvirus infections occur in juvenile brown kiwi, but the incidence of infection is low.
2. Rapid changes to lesions can indicate avipoxvirus – immediate isolation is recommended whilst awaiting confirmation.
3. Polymerase Chain Reaction (PCR) testing for avipoxvirus is optimal as histological testing can give false-negative results.
4. Aggressive debridement of pox lesions should be avoided, and biopsy sites should not be sealed after use.
5. In order to reduce transmission of the avipoxvirus, it is important to isolate infected birds and remove disposable consumables after use.
6. Avipoxvirus is very stable in the environment often rendering disinfection ineffective, however decontamination can be attempted using anti-viral disinfectant solutions, such as SteriGENE ®.

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A brief report on Cases 1 and 2 was presented by the author at the 2015 Conference (at Hamilton) of the Wildlife Society of the New Zealand Veterinary Association.

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