Clinical beak and feather disease virus infection in wild juvenile eastern rosellas of New Zealand; biosecurity implications for wildlife care facilities

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Abstract

CASE HISTORY: Four juvenile eastern rosellas (Platycercus eximius) were admitted to two separate wildlife care facilities in the Auckland region by members of the public. They had missing or dystrophic wing and tail feathers that rendered them flightless, suggestive of beak and feather disease virus (BFDV) infection. Two were subject to euthanasia after failing to re-grow their feathers, with samples taken for histopathology and PCR analysis. Blood samples were obtained from the other two birds at the time of examination, however these individuals were lost to follow up.

PATHOLOGICAL AND MOLECULAR FINDINGS: Basophilic inclusion bodies were observed in histological sections of the feather bulb, typical of BFDV infection, from the two euthanised individuals. Blood from all four birds tested positive by PCR for BFDV, and analysis of the recovered full BFDV genomes identified them as belonging to the BFDV-A strain.

DIAGNOSIS: Beak and feather disease virus infection.

CLINICAL RELEVANCE: This report highlights the clinical impacts of BFDV in juvenile eastern rosellas that may result in their admission to wildlife care facilities, creating a biosecurity risk in institutions that may host other native parrots intended for release. The environmental stability of BFDV and resistance to disinfection requires strict quarantine procedures to prevent contamination and spread within a facility. It is recommended that high-risk species such as wild eastern rosella be excluded from facilities that may also house native parrots.
Introduction

Beak and feather disease virus (BFDV) is a single stranded DNA virus that only infects parrots, with infection resulting in a range of clinical signs depending on the age and species of parrot infected (Pass and Perry 1984; Raidal and Cross 1995), as well as the host immune response (Borthwick 2005). Juvenile or nestling parrots show an acute and rapidly fatal form of the disease, with older parrots more likely to either overcome infection, or develop chronic disease characterised by dystrophic feathers, immunosuppression, and uncommonly beak lesions (Raidal 1995; Raidal and Cross 1995). The potential for clinically normal birds to develop a carrier status (Borthwick 2005) has important implications epidemiologically for maintenance and spread of the virus within a population or captive facility.

New Zealand is host to eight native parrots across three families, including two critically endangered species, the kakapo (Strigops habroptilus) and the orange fronted parakeet (Cyanoramphus malherbi) (Forshaw 2010). Surveillance has shown BFDV to be endemic in the introduced eastern rosella (Platycercus eximius) that have colonised the North Island of New Zealand (Ha et al. 2007; Massaro et al. 2012), but there is limited peer-reviewed published information on the presence of clinical disease in this species in captivity or the wild. Clinical disease can render affected individuals flightless, and this may result in their collection by concerned members of the public and admission to a variety of facilities, including wildlife care or rehabilitation centres, veterinary clinics and zoological institutions. The biosecurity implications of admitting to these facilities a species that has a high prevalence of BFDV infection in the wild, particularly where clinical signs are evident, are of particular concern as these same institutions may also treat or house native parrots intended for release to the wild.
Case history

Four juvenile eastern rosellas with missing or dystrophic wing and tail feathers were examined on-site at two wildlife rehabilitation facilities in the Auckland region of New Zealand in April 2012. All birds had been admitted as fledglings or juveniles to the care facilities between January and February 2012, and had failed to re-grow normal wing or tail feathers during the months preceding veterinary examination. Birds from the first site were identified as OR02 and OR03, and those from the second site as OR04 and OR05. A physical examination was performed to assess the health and welfare status of these birds, and samples were obtained for diagnostic purposes. Given the poor prognosis for return to normal feathering, and biosecurity risks of maintaining suspected BFDV-positive birds in a rehabilitation facility, birds OR04 and OR05 were subject to euthanasia and a full post mortem examination performed. Birds OR02 and OR03 were held pending results of the diagnostic tests, however were lost to follow up at the time of diagnosis.

Clinical findings

All four birds had similar clinical signs consistent with BFDV infection affecting primarily the wing and tail feathers. These feathers were either absent, or stunted with retained feather shafts, pinching of feather tips, or bleeding into feather shafts (Figure 1). Ulcerated and thickened skin was noted on the wing tips. The contour feathers and beaks appeared normal. All individuals were alert and bright, in good general body condition, however were unable to fly.

Pathological Findings

Post-mortem findings for birds OR04 and OR05 were normal, with the exception of the findings described above. Samples of internal organs, skin with growing feathers, air sac and gastrointestinal tract were processed routinely for histopathology, stained with H&E and 4 µm sections were examined by light microscopy. The bursa of Fabricius was not identified macroscopically or microscopically, nor was any remnant visible in the gastrointestinal tract. The pulp cavity of growing feathers from both birds contained moderate to numerous infiltrates of heterophils, small to moderate
infiltrates of macrophages, and rare to small numbers of cells with multiple cytoplasmic basophilic globular inclusions (Figure 2). All other organs examined appeared normal.

**PCR analyses**

Approximately 0.1 mL of whole blood was obtained from the medial metatarsal vein of all four birds, placed on Whatmann's filter paper No.3, and stored at room temperature until processing in June 2012. DNA was extracted from the blood on filter paper using the iGenomic blood DNA extraction kit (Intron Biotechnology, Gyeonggi-do, Korea) and 4 µL of this was used as a template for PCR screening using KAPA Blood PCR Kit Mix B (KAPA Biosystems, Wilmington, USA) as described by Julian *et al.* (2012, 2013).

To recover the full viral genome from positive samples, 1 µL of the extracted DNA was non-specifically amplified by rolling circle amplification (RCA) using TempliPhi (GE Healthcare, Pittsburgh, USA), and this RCA product was used as template for PCR based recovery of the full genome as described previously (Varsani *et al.* 2011; Julian *et al.* 2012, 2013). The resulting PCR product was cloned into pJET1.2 vector (Fermentas, Pittsburgh, USA) and the plasmid sequenced by primer walking at Macrogen Inc. (Seoul, Korea). The sequence contigs were assembled using DNAMAN (version 7; Lynnon Biosoft, Pointe-Claire, Quebec, Canada), aligned using MUSCLE (Edgar 2004), and a maximum likelihood phylogenetic tree created using PHYML (Guindon *et al.* 2010). All branches with <85% aLRT branch support were collapsed using MESQUITE ver 2.75 (http://mesquiteproject.org/mesquite/mesquite.html).

Molecular tests for BFDV infection of the four eastern rosellas revealed that all were positive for BFDV. The four BFDV isolates were distinct from the isolates recovered from infected native red-fronted parakeets (*Cyanoramphus novaezelandiae*) on Little Barrier Island in the Hauraki Gulf region of New Zealand (Figure 3), and belonged within the BFDV-A strain, which has only been found on the North Island of New Zealand. BFDV-A2–A6 genotypes have previously been recovered from wild eastern rosellas from the Auckland region. The BFDV recovered from OR02 was part of the BFDV-A4 genotype, OR03 was part of the BFDV-A2 genotype, whereas the virus recovered from OR04 and
OR05 were part of BFDV-A₁ genotype (Figure 3). The GenBank accession numbers were KF467251, KF467252, KF467253 and KF467254 for birds OR02, OR03, OR04 and OR05, respectively. The four BFDV genomes determined in this study shared >97% pairwise identity.

Discussion

The results indicate that BFDV infection in juvenile eastern rosellas can lead to clinical signs of feather loss and an inability to fly, which will encourage their capture and admission to wildlife care facilities by members of the public. Supportive care may enhance survival, however may not necessarily lead to resolution of clinical signs nor prevent chronic disease. The pathological examination of OR04 and OR05 found no evidence of systemic changes described in the rapidly fatal acute form of disease (Raidal and Cross 1995). This is consistent with recovery from the acute form of BFDV infection, which usually results in chronic disease with new feathers affected as moult progresses, and immune system dysfunction increasing susceptibility to secondary infections (Raidal 1995). Failure to detect tissue from the bursa of Fabricius may indicate atrophy or depletion of this lymphoid organ in association with the disease (Todd 2000). Whilst all birds were bright and in adequate general body condition, release was not recommended for welfare reasons including risk of predation in the wild, and likely progression of disease with shedding of virus into the environment, placing other parrots in the region at risk of BFDV exposure.

Recombination is common within BFDV genomes (Varsani et al. 2011; Julian et al. 2013), which may enhance virulence. Viral evolution and adaptive changes where populations mix can result in increased mortality, as occurred in the endangered Echo parakeet (Psittacula echo) in Mauritius during a BFDV outbreak from 2005 to 2006 (Kundu et al. 2012). Recombination is a specific risk for captive facilities that may bring strains and species into artificially close proximity, and subsequently release individuals back to the wild carrying novel variants of the virus. Full genome sequencing of BFDV-positive samples will therefore remain important to further understand the source and flow of
BFDV strains and variants within parrot populations of New Zealand, particularly if outbreaks occur in the future.

Wildlife care facilities in New Zealand must maintain strict biosecurity protocols to prevent BFDV infection and other diseases from entering and spreading within facilities, potentially leading to outbreaks of disease if individuals are released back to the wild. This is best achieved by separate quarantine facilities for new parrot admissions, with dedicated equipment and appropriate screening for BFDV. In New Zealand, diagnosis is only possible by PCR of blood, feathers or tissue. Factors that must be considered include uncertainty over the incubation period and reported variation over time for PCR positive results from affected birds (Borthwick 2005; Ha et al. 2009). A quarantine period of up to 6 months with testing every 90 days has been recommended to overcome these uncertainties (Borthwick 2005). Shedding of BFDV in affected individuals is considerable, and the virus is highly stable for multiple years in the environment as well as being resistant to disinfection (Todd 2000). Thus, effective decontamination of a facility once the virus is introduced is extremely difficult. A BFDV prevalence of up to 22.9 (95% CI=9.9–42.3)% has been reported in clinically normal wild eastern rosella in New Zealand (Massaro et al. 2012), making this species high risk for contamination of facilities, regardless of clinical presentation. With the described feasibility issues of providing appropriate quarantine and screening for BFDV for new admissions, it is not recommended they are kept on the same premises as native parrots intended for release.

The threat of BFDV spread in the wild from eastern rosellas to native parrots has been documented (Ha et al. 2007; Massaro et al. 2012), however this report identifies another important pathway for transmission within and between species in wildlife care facilities. Of particular importance is the risk of mixing viral strains and the release of new strains into the wild, as well as the issues associated with appropriate quarantine and screening for BFDV.
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Figure 1. Photograph of the wing of an eastern rosella (OR04) showing ulcerated wing-tip and dystrophic feathers, consistent with signs of infection with beak and feather disease virus.
Figure 2. Photomicrograph of a section of feather from an eastern rosella (OR05) showing basophilic viral inclusion bodies (arrows), typical of infection with beak and feather disease virus. (H&E, bar=20 µm).
Figure 3. Un-rooted maximum likelihood phylogenetic tree of the full genomes of 188 beak and feather disease virus isolates from all over the world showing their relationships and their strain demarcations (letters A–Z). The insert shows details of genotypes for the four eastern rosella (OR02–OR05) isolates described in this study that all belonged to the BFDV-A strain. Branches with <85% approximate likelihood ratio test (aLRT) support for branches have been collapsed, those with 85–94% aLRT support are marked with an open circle whereas those with ≥95% aLRT are marked with closed circles. Branches are coloured based on country of sampling as denoted in the key. AU = Australia; CN = China; DE = Germany; JP = Japan; NC = New Caledonia; NZ = New Zealand; PL = Poland; PT = Portugal; TH = Thailand; UK = United Kingdom; US = United States of America; ZA = South Africa; ZM = Zambia.