

Communication

# A PCR-Based Retrospective Study for Beak and Feather Disease Virus (BFDV) in Five Wild Populations of Parrots from Australia, Argentina and New Zealand

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**Abstract:** The beak and feather disease virus (family *Circoviridae*) is a virus of concern in the conservation of wild Psittaciformes globally. We conducted a PCR screening for the beak and feather disease virus (BFDV) using samples collected during previous field studies (1993–2014) in five populations of parrots of the Southern Hemisphere: Eclectus parrots (*Eclectus roratus*) and Crimson rosellas (*Platycercus elegans*) from Australia, Burrowing parrots (*Cyanoliseus patagonus*) and Monk parakeets from Argentina (*Myiopsitta monachus*), and Forbes' parakeet from New Zealand (*Cyanoramphus forbesi*). A total of 612 samples were screened. BFDV was not detected in any of the sampled birds. Our results provide a retrospective screening, covering three different tribes of Old and New World parrots, including two of the most numerous species, and contributing a large set of negative results. Furthermore, our results suggest that geographical and temporal differences in BFDV distribution may exist and merit further research, as a critical component in the efforts to manage the disease and its epidemiological aspects. The results presented here hold the potential to provide a baseline for future studies investigating the temporal evolution and the spread of BFDV.

**Keywords:** BFDV; *Circoviridae*; infectious disease; Psittaciformes; surveillance; viral infection; vulnerable taxa; wild populations

## 1. Introduction

Existing and emerging pathogens can drive rapid changes in population numbers and in the genetic diversity of the wild host population [1]. Pathogens have caused declines in previously large populations or even increased the rate of decline in endangered species [2–4]. Moreover, global pet trade and climate changes hold great potential to extend

current pathogen distributions and need to be considered as potential risk factors for the introduction of disease to wildlife [5–7]. For this reason, infectious disease has become a major challenge for conservation; thus, knowledge of the extent of infectious diseases in wildlife populations has become increasingly important for conservation work [8,9].

Parrots and cockatoos (Psittaciformes) have long been recognized as one of the most threatened orders of birds globally, with nearly a third of all known species classified as ‘at risk of extinction’, and a larger number facing population decline [10,11]. There are multiple factors associated with declining parrot populations, however, capture of wild parrots for the pet trade, intensified agriculture, hunting, and logging are the most frequent threats [10,11], with depredation by introduced species being a serious threat on islands [12]. Moreover, susceptibility to diseases substantially threatens some parrots e.g., Philippine cockatoo *Cacatua haematuropygia*, Cape parrots (*Poicephalus robustus*), blue-headed racquettail *Prioniturus platenae*, orange-bellied parrot *Neophema chrysogaster* [13–15].

The potentially negative effects of diseases for the survival of endangered parrots have been widely acknowledged [11,16,17] and have triggered abundant research. Studies on diseases, health and pathogens of captive parrots are published regularly [15,18,19]. Nevertheless, there is limited information on pathogenic infection in free-living Psittaciformes [20–29]. This paucity of studies on pathogens and diseases among free-living parrots makes it clear that we only partially understand their role as a threatening factor.

The beak and feather disease virus (BFDV) is a small circular single stranded DNA virus in the family *Circoviridae* [30,31], often cited as a pathogen of conservation concern for parrots in the wild, as well as in captivity [6,8,29,32], given its immune-suppressive effect in infected birds [33,34]. Abnormal plumage and morphological development, anaemia, damage of the lymphoid tissue, feather loss and weight loss among infected birds are common symptoms associated with this viral infection [35].

BFDV infects predominantly Psittaciformes [35], and is reported to cause high mortalities in avicultural collections [36] and in at least two free-living populations [37–39]. Recent evidence indicates, however, that BFDV can also infect non-parrot species [40]. In general, the virus has been reported as infecting over 10% of known parrot species, a figure that comes mostly from studies on captive birds [8,18,41,42]. Despite a wealth of information on captive birds (e.g., [18,41,43,44]), the prevalence of the virus in wild populations remains largely unknown for most regions except Australia, Mauritius, New Caledonia and New Zealand [8,26–28,42,45–49].

The advances in molecular techniques to detect the virus (e.g., [28,46,50]) open up an opportunity to conduct large scale surveys for BFDV among wild populations of Psittaciformes, and especially to screen large collections of blood samples from long term studies on parrots. Here, we present a retrospective study investigating the presence of BFDV among five wild populations of Psittaciformes belonging to three different tribes: (a) Psittaculini, the Eclectus parrot (*Eclectus roratus*) from tropical Australia, (b) Platycercini, the Crimson rosella (*Platycercus elegans*) from temperate Australia, and the Forbes’ parakeet (*Cyanoramphus forbesi*) from the Chatham Islands, New Zealand, and (c) Arini, the Burrowing parrot (*Cyanoliseus patagonus*) from the Patagonian steppes and Monk parakeet (*Myiopsitta monachus*) from Central Argentina.

## 2. Methods

We used 612 blood samples collected during previous studies (Table 1), to investigate the presence of BFDV. Details on the sample and populations sizes for each species are given in Table 1. Every individual was sampled once.

**Table 1.** Details on blood samples from five wild populations of Psittaciformes in this study.

Species	Estimation of Population Size	Reference for Population Size	Year of Sample Collection	Blood Samples (n)		Total
				Adult	Nestling	
<i>Eclectus roratus</i>	3000	Psittaculini [51]	1997–2007	24	291	315
<i>Platycercuselegans</i>	550	Platycercini [52]	1993–1995	17	52	69
<i>Cyanoramphus forbesi</i>	1000	[53]	2014	95	–	95
<i>Cyanoliseus patagonus</i>	75,000	Arini [54]	December 1998, December 1999	49t	55	104
<i>Myopsitta monachus</i>	500	[55] and E.H.B. unpubl. data	December 2000	29	–	29

Samples from *Eclectus* parrots were taken over the course of a long-term study (1997–2007) on Cape York Peninsula in northern Queensland Australia (12°45' S, 143°17' E) [56,57]. Most samples were taken from nestlings in nest hollows 15–25 m above the ground in rainforest trees. Adults were also captured using mist nets set at similar heights in the rainforest canopy. Approximately 100 µL of blood was taken from the brachial vein of each captured individual. *Eclectus* parrot blood was stored in 70% ethanol [57,58].

Samples from Crimson rosellas were collected from adult and nestling birds breeding in Black Mountain Nature Reserve, Australian Capital Territory (35°16'28'' S, 149°05'55'' E) [52]. Birds were captured in nest-boxes between 1993 and 1996; a small blood sample (50 to 100 µL) was taken from the brachial vein of each individual, and preserved in Queen's Buffer (10 mM Tris, 10 mM NaCl, 10 mM disodium EDTA, 1% n-lauroylsarcosine, pH 8.0) [59]. Blood samples were taken from adults on capture and from nestlings between 25 and 30 days of age.

Forbes' parakeets were captured using mist-nets on Mangere Island, Chatham Islands (44°26' S, 176°29' W), in March 2014. Blood samples (200 µL) were taken by puncture of the brachial vein immediately after capture and preserved in Queen's Buffer [59]. Only adults were sampled.

Burrowing parrots were captured at its major colony in El Cóndor, north-eastern Patagonia, Argentina (41°04' S, 62°50' W) during regular nest inspections in December 1998 and December 1999 [54]. Adults were sampled when found in the nest; nestlings were sampled between the age of 38 and 60 days. Monk parakeet samples were obtained in an area of 600 ha, situated near Jesús María, Córdoba, Argentina (31°05' S, 64°11' W) [55]. Monk parakeets were captured in their nests during December 2000. Blood samples (200 µL) of the adult and nestling burrowing parrots, as well as of adult monk parakeets, were taken by puncture of the brachial vein immediately after capture. The blood was stored in 70% ethanol [58].

In 2014, DNA was extracted from 10 µL of blood, which was added to 10 µL of 'lysis solution' from the Extract-n-Amp™ Blood PCR Kit (Sigma-Aldrich, St Louis, MO, USA) and incubated for 10 min at room temperature. Ninety microliters of this kit's 'neutralization solution' was subsequently added to yield crude total DNA. One microliter of the crude extract was used as template in the subsequent PCR [46]. Extracted DNA was stored at −20 °C. In addition, in 2014, as described in previously published studies [18,46,47,60], BFDV specific PCR screening was carried out using KAPA Blood PCR Kit Mix B (KAPA Biosystems, Wilmington, DE, USA) using the primer pair forward 5'-TTAACAACCCTACAGACGGCGA-3' and reverse 5'-GGCGGAGCATCTCGCAATAAG-3', which target a 605 bp region of the *rep* gene of BFDV [61]. The reaction volume was 25 µL with 1 µL of 10 µM F/R primer pair, 12.5 µL of the 2xKAPA Blood PCR Kit Mix, 1 µL of DNA templates and 10.5 µL of sterile molecular grade water. The PCR program contained an initial step of 94 °C for 5 min, which was followed by 25 cycles of 94 °C for 30 s, 56 °C

for 30 s and 72 °C for 45 s and with a final 1 min extension step at 72 °C and cooling to 4 °C for 10 min. DNA from a BFDV-infected red-fronted parakeet (*Cyanoramphus novaezelandiae*) from Little Barrier Island was used as a positive control [62]. The total DNA used as positive control was extracted from 60 µL of blood using the Qiagen QIAamp DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's protocols.

### 3. Results

We did not detect BFDV in any of the blood samples investigated by PCR.

### 4. Discussion

Surveillance for pathogens is a fundamental element for understanding the temporal and spatial prevalence of wildlife diseases and for understanding transmission pathways and effects on animal populations [63]. We applied a commonly used PCR screen [18,46,47,60] to detect viral DNA in blood samples collected during previous field studies of Eclectus parrots, Crimson rosellas, Forbes' parakeets, Burrowing parrots and Monk parakeets. Our negative results suggest that BFDV was not present in the studied populations at the time of sampling, and show some differences with previous studies, which could be related to temporal, geographical and captive versus wild population differences in BFDV prevalence and distribution. BFDV has previously been reported from captive Eclectus parrots [45,64,65]; however, the wild population here investigated is isolated from large human populations and parrots kept in captivity. Free-ranging Crimson rosellas on Norfolk Island and in Victoria, Australia, have been reported with BFDV [26–28,66], yet the samples in the current study originate from a population within and surrounding the city of Canberra, where a previous BFDV study found a very low number of potentially infected individuals [67]. BFDV has been reported on close relatives of Forbes' parakeets, including red-fronted parakeets and yellow-crowned parakeets (*Cyanoramphus auriceps*) [46], but has not been detected in other *Cyanoramphus* species in the wild. For Monk parakeets, the virus has been found in 37% of sampled individuals belonging to a feral population in Spain [68]. This high prevalence could be related to the origin of the birds, which accidentally escaped from captivity, where BFDV has been reported frequently [8,18,36]. To our knowledge, BFDV infection in Burrowing parrots is unknown for either captive or free-living individuals.

There are an increasing number of field studies with Psittaciformes worldwide; commonly, blood samples are collected. Those samples could be used to increase the range of species screened in the wild, allowing for a better understanding of the geographical distribution of BFDV. Moreover, Fogell et al. [8] pointed out that two biases currently exist in BFDV research, namely, the lack of (1) research in regions of the world such as South America and Southeast and Southern Asia, both characterised by a high parrot diversity, and (2) publications reporting negative results. Recent studies are starting to fill those gaps. Vaz et al. [29] using pathogen-specific PCR, evaluated the presence of BFDV. As in our study, Vaz et al. [29] detected no BFDV DNA in a large sample of 205 wild nestlings and 90 nestlings from the illegal trade. Moreover, we are confident that our study also makes a substantial contribution to BFDV research by providing further screening results for South American parrots, including two of the most numerous species, and by contributing a large screening with negative results, obtained with a methodology thoroughly tested [18,46,47,60]. Furthermore, our results suggest that geographical differences in BFDV distribution may exist and merit further research, as a critical component in the efforts to manage the disease and its epidemiological aspects. Lastly, the results presented here hold the potential to provide a baseline for future studies investigating the temporal evolution and the spreading of BFDV. However, two final cautionary remarks are needed. First, we acknowledge that there is a possibility that the nucleic acid may be damaged in storage and transport; this may impact the amplification of the target virus sequences in some of the samples. Second, the widely applied PCR protocol [18,46,47,60] used in this study has some limitation. BFDV is known for a high genetic diversity [68–70]; it cannot be

fully excluded that the primers used in this investigation might have missed some genetic variants. Thus, future studies should evaluate the presence of the virus based on any previous identification BFDV sequences from these hosts in captivity or introduction on new regions. Nonetheless, the primer pair we have used in this study binds with 100% complementarity to a BFDV sequence (GenBank Accession # MT303064) derived from the blood sample of Monk parakeets in Spain [68].

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**Institutional Review Board Statement:** The Eclectus parrot research was conducted under license from the Queensland Government and the ANU Animal Ethics Committee (Permit No: C.R.E.35.04). Crimson Rosellas sampling was conducted in accordance with ANU Animal Ethics guidelines (ANU Animal Ethics Permit J.BTZ.22.93), an ACT Parks Capture and Release Permit LT96023, and an ABBBS banding permit 1778. Forbes’ parakeets sampling was approved by the Department of Conservation, 19621-FAU, New Zealand. Burrowing Parrot sampling was carried out under permission of the Dirección de Fauna de la Provincia de Río Negro, Argentina (143089-DF-98). Monk parakeet sampling permit was granted by the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (CONICET) to E.H.B.

**Data Availability Statement:** All data are available in the main text.

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