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Short Communication

Preliminary surveillance for beak and feather disease virus in wild parrots of New Caledonia: implications of a reservoir species for Ouvea Parakeets

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Abstract. *Beak and feather disease virus* (BFDV) is a recognised key threat for the conservation of parrots globally, causing morbidity and mortality of individuals in susceptible species. We present findings from a survey in 2012 to investigate the presence of BFDV in wild New Caledonian parrots, including the endangered Ouvea Parakeet (*Eunymphicus uvaeensis*). Blood and feather samples from seven Ouvea Parakeets and 13 New Caledonian Rainbow Lorikeets (*Trichoglossus haematodus deplanchii*), and feathers from 15 New Caledonian Rainbow Lorikeets, five Horned Parakeets (*Eunymphicus cornutus*) and six New Caledonian Parakeets (*Cyanoramphus saisseti*) obtained from passive sampling, were tested by polymerase chain reaction (PCR) for BFDV. We identified a BFDV prevalence of 25% (95% CI 11–45%) in wild New Caledonian Rainbow Lorikeets, suggesting this species may act as a reservoir for persistence of BFDV in the wild, placing other parrots in New Caledonia at risk. All other parrot species tested negative for BFDV. New Caledonian Rainbow Lorikeets were introduced to Ouvéa Island in the 1970s, potentially bringing BFDV with them. As Ouvea Parakeets are restricted to this small island, we strongly recommend surveillance screening for BFDV in this species to guide future biosecurity and conservation efforts, and further understand the risk posed by BFDV to threatened parrots.

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Introduction

The role of emerging infectious diseases in declines of wildlife is increasingly recognised, with dramatic examples including population crashes of a suite of amphibians owing to chytrid fungus (Skerratt *et al*. 2007), and the recent sweeping declines in North American bats from white nose syndrome (Foley *et al*. 2011). The historical introduction of avian malaria to the Hawaiian islands and the subsequent extirpation of several native species is one of the most high-profile extinction events said to be related to disease (Warner 1968), and in parrots there is increasing evidence of a significant global threat to vulnerable or small populations from *beak and feather disease virus* (BFDV), the agent of psittacine beak and feather disease (PBFD) (Peters *et al*. 2014). The inclusion of disease surveillance as part of

conservation management and research is therefore critical to ensure all threats to survival have been recognised and addressed.

BFDV was identified as a key threat to endangered parrots in 2001 under the Australian *Environment Protection and Biodiversity Conservation Act* 1999 (Commonwealth), leading to the development of a threat abatement plan in 2005 (Department of Environment and Heritage 2005). The virus caused significant mortality of the endangered Mauritius Parakeet (*Psittacula echo*) in 2006 (Kundu *et al*. 2012), is a conservation concern for wild Cape Parrots (*Poicephalus robustus*) in South Africa (Heath *et al*. 2004), and has recently re-emerged, causing disease, in a wild population of the critically endangered Orange-bellied Parrot (*Neophema chrysogaster*) in Australia (Peters *et al*. 2014). The virus evolves rapidly through high nucleotide substitution rates (~2.27×10^{-3} substitutions per site per year) (Harkins *et al*. 2014), which, coupled with extensive recombination (Varsani *et al*. 2011; Julian *et al*. 2013), means strains and altered virulence (i.e. ability for the virus to cause disease) can develop rapidly (Kundu *et al*. 2012; Julian *et al*. 2013; Sarker *et al*. 2014). BFDV is suspected to have originated in Australia, with movements of parrots by legal and illegal wildlife trade contributing to the global spread of the virus, and providing a contemporary avenue for dissemination of novel viral strains (Harkins *et al*. 2014). Captive parrot lineages of BFDV originating in Europe have been found in countries hosting wild native parrots, including New Caledonia (Julian *et al*. 2012), highlighting the plausible threat posed by distant, potentially more pathogenic strains of BFDV, through accidental or deliberate releases of captive parrots to the wild (Harkins *et al*. 2014).

BFDV can cause severe disease in a range of parrot species, including high mortality in juvenile birds and immune suppression and abnormal feather-growth in older birds (Raidal 1995). Failure to grow normal wing- and tail-feathers makes affected birds vulnerable to predation, so the impacts are not always direct and may be underestimated. However, complete recovery from the virus is also widely reported (Raidal *et al*. 1993; Raidal 1995; Todd 2000; Harrison and Lightfoot 2006; Tomasek and Tukac 2007), indicating varying degrees of host susceptibility based on exposure factors including virus type, host species, age and immune status.

BFDV has been detected in wild New Caledonian Rainbow Lorikeets (*Trichoglossus haematodus deplanchii*) (Julian *et al*. 2012), including clinically affected juvenile birds with failure to grow wing- or tail-feathers. In addition, BFDV has also been found in New Caledonian Parakeets (*Cyanoramphus saisseti*), Eclectus Parrots (*Eclectus roratus vosmaeri*), Red-rumped Parrots (*Psephotus haematonotus*) and Rose-ringed Parakeets (*Psittacula krameri*) held in captivity in New Caledonia (Julian *et al*. 2012). Susceptibility and outcomes of exposure to BFDV in the three endemic parrot species of New Caledonia in the wild are not known, although infection in captivity has been reported (Tomasek and Tukac 2007; Julian *et al*. 2012). This study conducted systematic surveillance for BFDV in New Caledonian parrots in different locations, to investigate the spatial distribution of virus in the wild, and to provide ongoing surveillance of viral strains circulating in the New Caledonian Rainbow Lorikeet population. The low population densities and patchy geographical spread of the three parrot species endemic to New Caledonia may not be sufficient to maintain or spread virus throughout their distribution, but Rainbow Lorikeets are abundant and distributed throughout the main island (Legault *et al*. 2011), which could enable persistence of BFDV and viral flow. New Caledonia provides an opportunity to study the multi-species interactions and viral flow between a discrete set of parrot species, and to provide further empirical evidence for understanding the conservation implications of this pathogen for parrots globally.

A secondary aim of the study was to engage a wide range of stakeholders, including government agencies, members of the community, local non-government organisations and researchers to: (1) raise awareness of the potential for disease to act as a conservation threat, especially in small or declining populations; and (2) encourage incorporation of appropriate disease surveillance as part of conservation management planning for New Caledonian parrots. Building awareness can enhance the acquisition of samples through passive surveillance mechanisms (e.g. where sampling design is not targeted to disease surveillance, and samples are acquired as part of other studies or through wildlife rescues), which is a cost effective, although biased, method for obtaining disease surveillance data (Duncan *et al*. 2008).

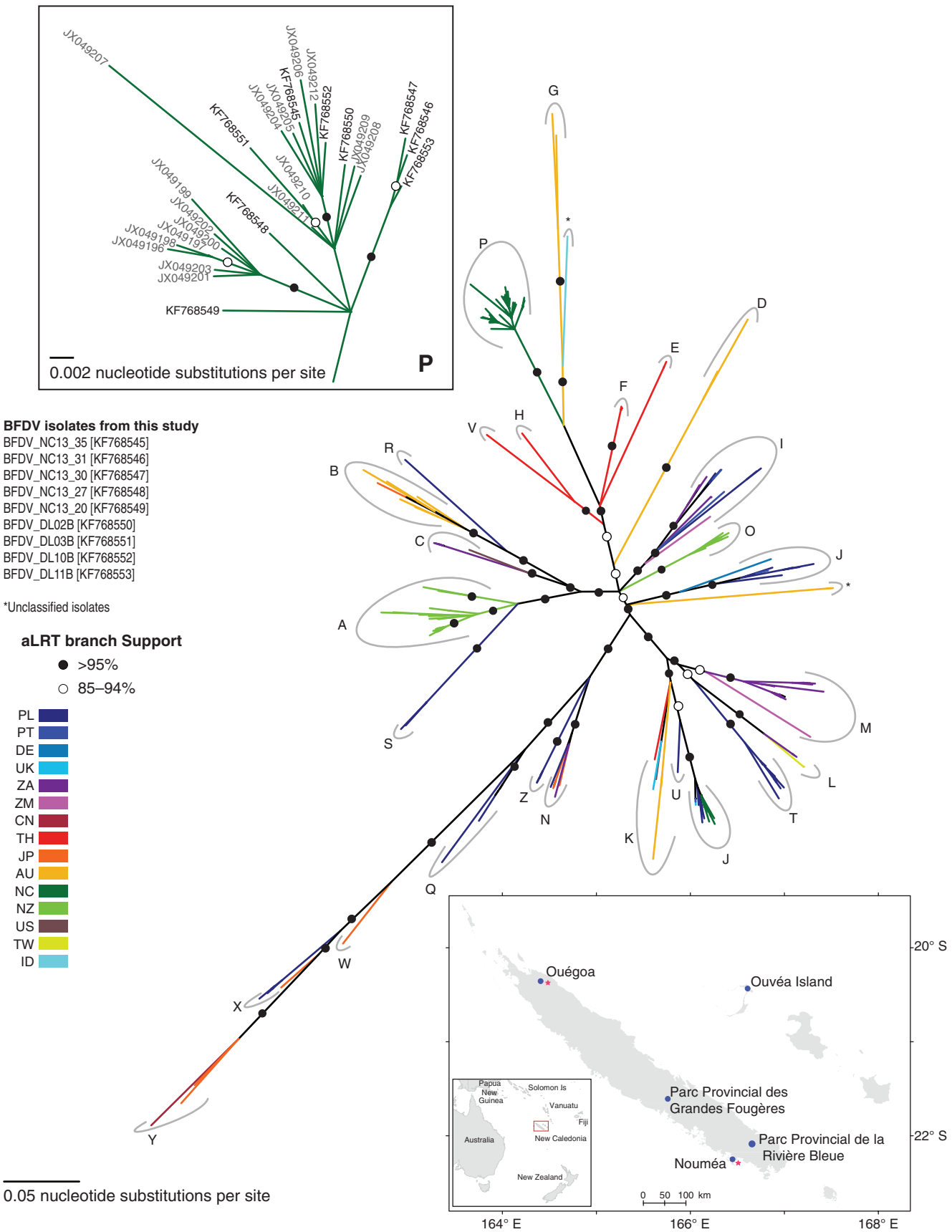
Methods

Sampling locations and study species

New Caledonia (21°11′S, 165°12′E) is an archipelago in the Melanesian subregion of the south-western Pacific Ocean, comprising the main island of Grande Terre and many outlying islands, such as Ouvéa in the Loyalty Islands. Three of the four extant species of New Caledonian parrots are endemic to the archipelago. The Horned Parakeet (*Eunymphicus cornutus*) and New Caledonian Parakeet are patchily distributed across Grande Terre (Legault *et al*. 2011), and listed as vulnerable globally (BirdLife International 2013*a*, 2013*b*), whereas the Ouvea Parakeet (*Eunymphicus uvaeensis*) is restricted to Ouvéa Island (Barré *et al*. 2010; Legault *et al*. 2013) and classified as endangered (BirdLife International 2013*c*). The New Caledonian Rainbow Lorikeet is an endemic subspecies and widely distributed throughout New Caledonia (Legault *et al*. 2011), including an introduced population on Ouvéa Island (Barré *et al*. 2010).

We captured parrots with mist-nets over 2 weeks in April 2012 (15–30 April). We chose netting sites based on the presence of parrots and feasibility of captures. We captured birds at one site on Ouvéa Island (20°26′S, 166°36′E) and three sites on Grande Terre: Ouégoa (20°20′51″S, 164°25′54″E), Parc Provincial des

Fig. 1. Maximum likelihood phylogenetic tree of the full genomes of BFDV isolates from all over the world, showing their relationships and strain demarcations (letter A–Z). Branch colour indicates country of sampling, as denoted in the key using a standard two-letter international country code (PL, Poland; PT, Portugal; DE, Germany; UK, United Kingdom; ZA, South Africa; ZM, Zambia; CN, China; TH, Thailand; JP, Japan; AU, Australia; NC, New Caledonia; NZ, New Zealand; US, United States ; ID, Indonesia). The enlarged insert (P) provides details of the seven wild and two captive New Caledonian Rainbow Lorikeet isolates described in this study from the BFDV-P strain, including GenBank accession numbers. Map of New Caledonia on the lower right highlights areas sampled including wild passive surveillance samples (blue dots), and locations with positive samples (red stars).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JX049206
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JX049199
JX049202
JX049200
JX049197
JX049198
JX049196
KF768548
JX049203
JX049201
KF768549
0.002 nucleotide substitutions per site
P
BFDV isolates from this study
BFDV_NC13_35 [KF768545]
BFDV_NC13_31 [KF768546]
BFDV_NC13_30 [KF768547]
BFDV_NC13_27 [KF768548]
BFDV_NC13_20 [KF768549]
BFDV_DL02B [KF768550]
BFDV_DL03B [KF768551]
BFDV_DL10B [KF768552]
BFDV_DL11B [KF768553]
*Unclassified isolates
aLRT branch Support
>95%
85–94%
PL
PT
DE
UK
ZA
ZM
CN
TH
JP
AU
NC
NZ
US
TW
ID
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O
J
A
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Z
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U
T
K
J
Q
W
X
Y
0.05 nucleotide substitutions per site
Ouégoa
Ouvéa Island
Parc Provincial des Grandes Fougères
Parc Provincial de la Rivière Bleue
Nouméa
Papua New Guinea
Solomon Is
Vanuatu
Fiji
New Caledonia
Australia
New Zealand
0 50 100 km
20° S
22° S
164° E
166° E
168° E

Grandes Fougères (21°36′47″S, 165°45′56″E) and the city of Nouméa (22°17′34″S, 166°26′58″E) (Fig. 1).

We used both standard mist-nets attached to metal poles (9 × 3 m, 30-mm mesh) and aerial mist-nets suspended in the canopy by ropes (12 × 9 m, 38-mm mesh) (Avinet, Dryden, NY, USA) to capture parrots. We caught New Caledonian Rainbow Lorikeets at all three sites on Grande Terre and Ouvea Parakeets only on Ouvéa. We were unable to catch adult Horned Parakeets, and we did not try to catch adult New Caledonian Parakeets because their population densities were low at the capture sites.

We also obtained feather samples from 26 wild parrots from the Parc Provincial de la Rivière Bleue (22°5′53″S, 166°39′50″E), Nouméa and the Parc Provincial des Grandes Fougères. We obtained feathers from four adult and one nestling Horned Parakeets and six nestling New Caledonian Parakeets handled as part of other research projects (conducted 2008–13). The four adult Horned Parakeets were killed by predators, and the seven nestlings were captured in their nests ~1 week before they fledged. We also tested feathers from 15 wild adult New Caledonian Rainbow Lorikeets brought to the Parc Zoologique et Forestier (PZF) in Nouméa between 2012 and 2013. Two captive New Caledonian Rainbow Lorikeets from PZF were also tested as part of this study but were not included in analyses for wild prevalence of BFDV (and are not included in Table 1). All feathers were either obtained from frozen stored specimens of birds found dead or removed by researchers from live birds during handling events or on submission to the PZF.

Sampling

Mist-netted Ouvea Parakeets were anaesthetised with isoflurane using a portable field anaesthetic machine (Advanced Anaesthesia Specialists, Sydney, Australia), to enable detailed physical examinations, photography of birds, taking of measurements, and sampling and banding while minimising stress to the birds. Mist-netted New Caledonian Rainbow Lorikeets were sampled conscious using a simplified protocol (cf. that for Ouvea Parakeets) and several secondary wing-feathers were clipped to prevent re-sampling of individuals. For both species, blood was obtained from the medial metatarsal vein using a 1-mL syringe and 25-gauge needle, placed on Whatmann number 3 filter paper and air-dried before storage at room temperature. Growing feathers (blood-feathers) were extracted and placed in sealable plastic bags and stored at room temperature.

Molecular analyses

We extracted DNA from blood on filter paper and feathers using the iGenomic blood DNA extraction kit (Intron Biotechnology, Gyeonggi-do, South Korea) according to manufacturer's protocols. To screen for BFDV we used 4 µL of the extracted DNA as a template for BFDV polymerase chain reaction (PCR), with primers targeting an ~605 nucleotide region of the replication associates protein (Rep) gene (5′-TTA ACA ACC CTA CAG ACG GCG A-3′ and 5′-GGC GGA GCA TCT CGC AAT AAG-3′) (Ritchie *et al*. 2003) and the KAPA Blood PCR Kit Mix B (Kapa Biosystems, Wilmington, MA, USA), as previously described by Julian *et al*. (2012, 2013).

For BFDV-positive samples, we enriched 1 µL of the extracted DNA for circular DNA using TempliPhi (GE Healthcare, Pittsburgh, PA, USA) as previously described (Julian *et al*. 2012; Julian *et al*. 2013; Varsani *et al*. 2011). To recover the full genomes, 1 µL of the enriched DNA was used as a template for PCR amplification with abutting (back-to-back) primers (BFDV-AV-F 5′-CYT ACY CTK GGC ATT GTG GC-3′, BFDV-AV-R 5′-TAT HAC RTC BCC YTC YTT ACT GCA-3′) and KAPA HiFi HotStart DNA polymerase (Kapa Biosystems). We cloned the amplified full genomes into pJET1.2 vector (Thermofisher, Waltham, MA, USA) and sequenced the recombinant plasmid by primer walking at Macrogen Inc. (Seoul, South Korea).

Sequences were assembled using DNAMAN version 7 (Lynnon Biosoft, Pointe-Claire, QC, Canada). The full genomes recovered from this study were aligned with those available in public databases using MUSCLE (Edgar 2004). PHYML (Guindon *et al*. 2010) was used to infer a maximum likelihood phylogenetic tree on the resultant alignment incorporating the GTR+I+G4 nucleotide substitution model, selected as the best substitution model by jModelTest (Posada 2008), with approximate likelihood ratio test (aLRT) branch support. All branches with <85% aLRT branch support were collapsed using MESQUITE version 2.75 (Maddison and Maddison 2011).

Table 1. Estimates of BFDV prevalence and likelihood of detection of disease in wild New Caledonian parrots

Probabilities of disease detection are only provided for those species where disease was not detected, to indicate the likelihood of finding an infected individual in this species given the sample size. PGF, Parc Provincial des Grandes Fougères; PZF, Parc Zoologique et Forestier (captive); PPRB, Parc Provincial de la Rivière Bleue

Common name	Species	Locations sampled	Number of samples collected in April 2012 (number positive)	Number of passive samples (number positive)	Total number sampled (total positive)	Prevalence BFDV (95% CI)	Probability of disease detection
New Caledonian Rainbow Lorikeet	*Trichoglossus haematodus deplanchii*	Nouméa	4 (2)	15 (3)	28 (7)	25% (11–45%)	NA
		PGF	5 (0)	0			
		Ouégoa	4 (2)	0			
Horned Parakeet	*Eunymphicus cornutus*	PPRB	0	3 (0)	5 (0)	0% (0–34%)	0.56
		PGF	0	2 (0)			
Ouvea Parakeet	*Eunymphicus uvaeensis*	Ouvéa Island	7 (0)	0	7 (0)	0% (0–41%)	0.47
New Caledonian Parakeet	*Cyanoramphus saisseti*	PPRB	0	5 (0)	6 (0)	0% (0–31%)	0.59
		PGF	0	1 (0)			

Epidemiological analyses

We calculated the likelihood of detection of disease for any species that tested negative using Epitools (Sergeant 2014). Assumptions for these calculations included a test sensitivity (probability of a true positive) of 0.9, a design prevalence (predicted prevalence of disease in a population) of 0.1, and population sizes reported in Legault *et al*. (2013). A test specificity (probability of a true negative) of 1 was assumed as full viral genomes were recovered from all positive results thereby precluding the possibility of a false positive. We did not calculate prevalence for each location (combining species) as it is not known if there is sufficient host susceptibility and viral flow between the different parrot species to warrant treating these as a metapopulation. However, as New Caledonian Lorikeets are continuously distributed and abundant across the main island of Grande Terre, it was considered appropriate to report a global prevalence for this species.

Results

We captured seven Ouvea Parakeets and 13 New Caledonian Lorikeets at four mist-netting sites during April 2012 (Fig. 1). There was no evidence of clinical BFDV infection on physical examination of the birds. All five Horned Parakeets, and six New Caledonian Parakeets obtained through passive sampling were in normal body and feather condition. The reason the New Caledonian Rainbow Lorikeets (n = 15) were brought to the PZF and any clinical signs on admission were not always recorded and so symptoms of BFDV cannot be excluded.

A total of 46 wild birds of the four native parrot species were tested for BFDV, either in both blood and feather samples (n = 20) or in feathers alone (n = 26) (Table 1). Prevalence of BFDV infection in all 28 wild New Caledonian Rainbow Lorikeets was 25% (95% CI 11–45%), with no positives detected in the three other parrot species (Table 1). Excluding the 15 passively acquired samples of New Caledonian Rainbow Lorikeets, which may introduce sampling bias towards a higher prevalence, the prevalence of BFDV infection in the remaining 13 Rainbow Lorikeets was 31% (95% CI 9–61%), a result that is not significantly different from the overall reported prevalence (z-test, P = 0.69).

The recovered full genomes of the BFDV from New Caledonian Rainbow Lorikeets belong to the same strain as that previously detected by Julian *et al*. (2012). Positive samples included two from the Ouégoa region (DL02B, DL03B), five from wild individuals of the Nouméa region (DL10B, DL11B, NC13–20, NC13–27, NC13–34) and two captive individuals from PZF (NC13_30, NC13_31) (Fig. 1).

Discussion

The PCR prevalence of BFDV in wild New Caledonian Rainbow Lorikeets assayed in this study was consistent with the high end of those reported in wild parrots elsewhere (range 15–28%) (Ha *et al*. 2007; Ortiz-Catedral *et al*. 2009: Massaro *et al*. 2012). The reported prevalence did not change significantly when the potentially biased samples from birds brought to the PZF were excluded from the analysis. This high prevalence of infection without clinical signs probably reflects a reservoir species capable of maintaining and disseminating virus within an environment, as appears to be the case with Eastern Rosellas (*Platycercus eximius*) in New Zealand (Ha *et al*. 2007). BFDV is highly stable for years within the environment (Todd 2000), and is spread in faeces, feather-dander and crop secretions (Ritchie *et al*. 2000). Reservoir species provide an ongoing source of virus that may infect sympatric parrots regardless of whether these other species can maintain the virus within their own populations. Epidemiologically, abundant reservoir species are the most significant element in a system (Rhyan and Spraker 2010), as without them small populations are more likely to develop endemic or epidemic fadeout, whereby disease becomes extinct in a population owing to stochasticity or depletion of susceptible individuals respectively (Lloyd-Smith *et al*. 2005). Thus, identifying reservoir species is vital to understanding the disease ecology of BFDV in a given location, as demonstrated by the presumed transmission of virus from an unknown reservoir species in the recent emergence of BFDV in the Orange-bellied Parrot (Sarker *et al*. 2014).

New Caledonian Rainbow Lorikeets are sympatric with New Caledonian Parakeets and Horned Parakeets over most of their range, on Grande Terre. However, habitat and dietary studies have suggested resource partitioning between the three species, particularly between New Caledonian Parakeets and Horned Parakeets, and these two species are granivorous whereas New Caledonian Rainbow Lorikeets are primarily nectarivorous (Legault *et al*. 2011, 2012). The ecology of Horned Parakeets and New Caledonian Parakeets, coupled with their low population densities, means there is little potential for direct contact between them and New Caledonian Rainbow Lorikeets, which currently reduces risks of virus spread between Rainbow Lorikeets and the parakeets. The most likely transmission risk is sharing of contaminated nesting sites, which may remain infectious for years (Peters *et al*. 2014), although nest-sharing between species has not been observed in New Caledonia (Theuerkauf *et al*. 2009).

Failure to detect BFDV in wild Ouvea Parakeets, Horned Parakeets and New Caledonian Parakeets may be attributed to small sample sizes, low prevalence within the population, or a true absence of disease. A low prevalence of disease may reflect low species susceptibility to the virus, population density limitations on transmission, or high mortalities with birds removed from the population for sampling before detection. With their patchy distribution and low abundance, it is possible that parakeets in New Caledonia act as dead-end or spill-over hosts, which cannot maintain infection and thus require source virus from a reservoir to become infected (Rhyan and Spraker 2010). Loss of wing- and tail-feathers in association with BFDV infection has been documented in captive Horned Parakeets (Tomasek and Tukac 2007). The affected birds eventually recovered, although the loss of feathers reported during infection would render wild parrots incapable of flight and highly susceptible to predation. BFDV infection has also been reported in a clinically normal captive New Caledonian Parakeet (Julian *et al*. 2012), and a study in New Zealand found significant loss of feathers in infected wild individuals of the closely related Red-crowned Parakeet (*Cyanoramphus novaezelandiae*) (Ortiz-Catedral *et al*. 2009). These studies suggest infection and clinical disease is possible in all parrot species of New Caledonia.

Failure to detect BFDV infection in the Ouvea Parakeet is important, although small sample sizes mean that caution should be applied when interpreting this result. Ouvéa Island is classed as an Important Bird Area (Spaggiari *et al*. 2007), and the endemic and endangered Ouvea Parakeet is threatened by habitat loss, predation, and competition for nesting sites from European Honeybees (*Apis mellifera*) (Barré *et al*. 2010). Community-based measures to stop nest-poaching led to a significant population increase between 1993 and 2009 (Barré *et al*. 2010), suggesting nestling survival is critical for conservation of the species. A virus such as BFDV, with highest mortality in nestlings (Raidal 1995), should be considered a key threat for the long-term viability of this species. New Caledonian Rainbow Lorikeets were introduced to Ouvéa Island in the 1970s (Barré *et al*. 2010), potentially bringing BFDV with them. The population of Rainbow Lorikeets on the island is small, and does not currently overlap with that of the Ouvea Parakeets (Barré *et al*. 2010). Determining the BFDV status of both parrot species on the island is critical, as the Ouvea Parakeet may at this point meet known criteria that enhance the likelihood of extinction by disease, in particular, the presence of a reservoir species in the New Caledonian Rainbow Lorikeet, and a small population that is potentially naïve to BFDV (Peters *et al*. 2014).

Full genome analysis of virus recovered in this study provides evidence that wild New Caledonian Rainbow Lorikeets act as a reservoir species for a single BFDV strain. European lineages of BFDV, signalling multiple introductions, have been detected in captive parrots in New Caledonia (Julian *et al*. 2012), although these have so far not been detected in the wild populations. BFDV is a highly recombinant virus (Varsani *et al*. 2011; Julian *et al*. 2013), and the release of captive parrots with new viral strains can, theoretically, lead to recombinants with increased morbidity or mortality. Changes in circulating strains of BFDV led to a spike in clinical disease and mortalities in the endangered Mauritius Parakeet in 2006 (Kundu *et al*. 2012), and mutations in functionally important regions of the virus led to the recent outbreak in Orange-bellied Parrots (Sarker *et al*. 2014). Ongoing surveillance of BFDV strains circulating in wild Rainbow Lorikeets is critical to the detection of new or emergent strains that may lead to outbreaks of disease. This should be coupled with communication of risks to aviculturists to ensure accidental or deliberate releases are minimised.

Surveillance for disease threats in wildlife can be costly, logistically challenging and time-consuming (Duncan *et al*. 2008), factors that contributed to the lack of capture of wild Horned and New Caledonian Parakeets in this study. One method of reducing surveillance costs is adoption of passive surveillance (Nusser *et al*. 2008). Through raising awareness of BFDV in New Caledonia, researchers, veterinarians, members of the public and other relevant organisations are encouraged to submit appropriate samples from wild parrots, particularly those found dead or with symptoms of BFDV infection. By targeting parrots with clinical signs (e.g. risk based), this surveillance improves the likelihood of detecting virus in the wild (Nusser *et al*. 2008).

This study provides evidence of a relatively high prevalence of BFDV infection in wild New Caledonian Rainbow Lorikeets on the mainland, with the conserved circulating viral strain (BFDV-P) detected in this species suggesting a single introduction of virus to the wild that has potentially adapted to the host. We recommend further targeted and passive surveillance sampling for BFDV to determine the threat level this virus may pose to conservation of the three other parrots species, in which BFDV has not been detected in the wild. The failure to detect BFDV in the endangered and highly range-restricted Ouvea Parakeet, and unknown BFDV status of the introduced population of New Caledonian Rainbow Lorikeets on Ouvéa Island, makes this island a priority for further investigation. If BFDV is not detected in Ouvea Parakeet, there are significant biosecurity implications for testing and potentially removing New Caledonian Rainbow Lorikeet from the island before the population expands. If BFDV is present in Ouvea Parakeets, then studying this group over time could provide much needed empirical evidence on the effects and dynamics of the disease in a wild, range-restricted population (Kundu *et al*. 2012). Studies such as these will inform conservation managers globally of the true risks of BFDV infection for threatened parrots.

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