Association of Avian Veterinarians Australasian Committee Ltd.
Annual Conference 2015 23, 18-27

# Psittacine Beak and Feather Disease Virus in wild Orange-bellied Parrots

Shubhagata Das, Subir Sarker, Andrew Peters, Shane R. Raidal
School of Animal and Veterinary Sciences,
E.H. Graham Centre for Agricultural Innovation
Charles Sturt University,
Boorooma St, Wagga Wagga, New South Wales, 2678, Australia

Annie Phillips
Biodiversity Conservation Branch,
Department of Primary Industries, Parks, Water and Environment
DPIPWE, 134 Macquarie Street,
Hobart, Tasmania, 7001, Australia

Jade Forwood
School of Biomedical,
E.H. Graham Centre for Agricultural Innovation
Charles Sturt University,
Boorooma St,
Wagga Wagga, New South Wales, 2678, Australia

## Abstract

We report an outbreak of beak and feather disease virus (BFDV) due to a novel genotype in the last remaining wild population of the critically endangered Orange-bellied Parrot *(Neophema chrysogaster*). We used PCR to detect BFDV in the blood of psittacine beak and feather disease (PBFD) affected wild Orange-bellied Parrot nestlings in Tasmania. Phylogenetic analysis of complete BFDV genome sequence data from infected birds supported the positioning of this clade genetically close to previous outbreaks of BFDV in captive birds in Tasmania. The detection of distinct lineages of BFDV in the remnant wild population of orange-bellied parrots, consisting of fewer than 50 birds, suggests a role for other parrot species as a reservoir for infection by spillover into this critically endangered species.

## Introduction

Psittacine beak and feather disease (PBFD) is a chronic, debilitating and ultimately fatal disease of Psittaciformes primarily involving the integument, alimentary, and immune systems of affected birds (Pass and Perry, 1984). In most psittacine bird species chronic infection and the development of clinical signs are associated with high levels of morbidity and eventual mortality. The disease can be expressed either acutely, ranging from sudden death, or with a chronic prolonged course of feather dystrophy ultimately leading to mortality (Pass and Perry, 1984). In grass parrots such as *Neophema* and *Psephotus* species feather lesions may be subtle and best detectable by histopathologic examination. Affected birds are highly susceptible to secondary infections due to immunosuppression, and it is this aspect of the disease that can degrade flock health and immunologic fitness. The aetiologic agent of PBFD, beak and feather disease virus (BFDV), is a member of the *Circoviridae* and one of the smallest viruses known to exist in terms of both physicochemical and genetic characteristics (Bassami et al., 1998). Since BFDV has a relatively simple but compact circular single-stranded DNA (ssDNA) genome of approximately 2,000 nucleotides the small genome of the virus facilitates whole genome viral epidemiologic analysis (Bassami et al., 2001). Like RNA and other ssDNA viruses, BFDV is prone to a high rate of genetic mutation, although the Rep gene is relatively conserved which conveniently assists with diagnosing infection by PCR detection methods (Ypelaar et al., 1999). Within Psittaciformes, BFDV exhibits quasi-species characteristics with emerging geographic or host-specificity demonstrable within various clades (Varsani et al., 2010b) and the observed occurrence of closely related clades in highly divergent parrot species is evidence of host-switching or host-generalism in several BFDV lineages (Varsani et al., 2010b; Massaro et al., 2012; Harkins et al., 2014; Jackson et al., 2014; Peters et al., 2014; Sarker et al., 2014b, 2015).

PBFD has been long recognized in Australian wild birds (Powell, 1903; Layton, 1936; McOrist et al., 1984; Pass and Perry, 1984; Perry et al., 1991; Raidal et al., 1993a), with the first record of an epidemic

with PBFD-like syndrome occurring in Red-rumped Parrots (*Psephotus haematonotus*) from 1887-8 in the Adelaide Hills, South Australia, which was blamed for the almost complete extirpation of the species from that area for more than 20 years (Ashby, 1907). The dispersion of wild-caught Australian parrot species such as the budgerigar (*Melopsittacus undulatus*) since the early 1840's when this bird was also sometimes identified by the terms *Nanodes undulatus*, the shell parrot or simply the betsherrygah (Leichhardt, 1846) has most likely resulted in the global spread of PBFD as it now affects a wide range of psittacine species both in wild and captive populations worldwide ( Clout and Merton, 1998; Raidal et al., 1993b; Bassami et al., 2001; Ha et al., 2007).

In 2001 PBFD was listed by the Australian Government under the Environment Protection and Biodiversity Conservation Act (1999) as a key threatening process for endangered psittacine birds (Department of the Environment and Heritage, 2005), in particular the critically endangered orange-bellied parrot (*Neophema chrysogaster*). PBFD complicated attempts to establish the orange-bellied parrot captive-breeding program in 1985 (Brown, 1988) and the recent re-emergence of unique BFDV genotypes in this species is of ongoing concern (Peters et al., 2013; Sarker et al., 2014d). In 1995 PBFD was also confirmed in the Norfolk Island green parrot (*Cyanoramphus cookii*) probably as a result of transmission following the introduction of the Eastern Rosella (*Platycercus eximius*) to the island (Stevenson et al., 1995; Department of Sustainability Environment Water Population and Communities, 2013). In New Zealand, Mauritius, South Africa and Indonesia, PBFD is recognised as a threat to many psittacine bird species (Heath et al., 2004; Ha et al., 2007; Ortiz-Catedral et al., 2009). Whilst it is clear that captive neotropical parrots are susceptible to BFDV infection (Julian et al., 2013) the conspicuous paucity of unique BFDV genotypes from South American parrots is so far unexplained given the prominence of macaws, conures and Amazon parrots in the North American and European pet and aviculture trade. The less than ideal hygiene and husbandry conditions that were present during the expansion of the pet bird trade should have allowed ample exposure to BFDV admixing from a variety of sources as has clearly occurred in European countries (Julian et al., 2013). The most recent phylogenetic studies provide increasingly more evidence that BFDV has originated in Australasian and not African or South American Psittaciformes (Bassami et al., 2001; ; Varsani et al., 2010b; Julian et al., 2012; Massaro et al., 2012; Peters et al., 2014; Sarker et al., 2014a, 2014b; Raidal et al., 2015; Sarker et al., 2015).

The Orange-bellied Parrot is one of the world's most critically endangered birds (Brown, 1988; Drechsler, 1998; Orange-bellied Parrot Recovery Team, 2013) with fewer than 50 wild birds thought to exist. The species has been the subject of considerable conservation efforts over the past three decades including the management of a captive insurance population and release of captive-bred birds to bolster wild population (Orange-Bellied Parrot Recovery Team, 2013). PBFD was recognized as a disease of concern in the first National Recovery Plan for the Orange-bellied Parrot (Brown, 1988) because the establishment of the captive-breeding program in 1985 was set back by an outbreak of the disease. PBFD was likely present in founder birds collected as juveniles from the wild and the outbreak was thought to have been exacerbated by poor siting of the breeding facility at Bridgewater, Tasmania, which experienced cold, damp winters. However, the disease was controlled in 1989 when the aviaries were relocated to more a favorable climatic area (Taroona, Tasmania) when PBFD appeared to be no longer a problem. Although, PBFD was confirmed in one wild bird with clinical signs collected at Swan Island, Victoria. The first releases of captively bred birds to the wild were carried out in 1993 and have continued annually for most years since then. The use of improved facilities and routine testing for infection by PCR, haemagglutination and haemagglutination inhibition (HI) assays has been used successfully between 1994 and 2006 to manage and prevent the transmission of infection in captivity. However PBFD re-emerged in the captive flock in 2007 in several juvenile captive birds (Sarker et al., 2014d). Efforts to control the disease included euthanasia of clinically diseased birds and segregation of clinically normal but PCR positive or HI positive birds and serial retesting. The recent detection of two distinct lineages of BFDV in the remnant wild population of orange-bellied parrots, suggested a role for other parrot species as a reservoir for infection by spillover into this critically endangered species (Peters et al., 2014). In the present report we document BFDV genotypes detected in wild nestling orange-bellied parrots during routine surveillance of the 2014-2015 breeding season and discuss implications for the management of this disease in the species.

## Materials and Methods

As part of a larger monitoring program for PBFD in wild birds blood was taken from 23 wild Orange-bellied Parrots in Melaleuca, Tasmania during the 2014-2015 summer breeding season. Blood sampling included 22 nestling (n=19 in 12 nest hollows) or juvenile (n=3) and one adult bird capture at feed tables. PBFD diagnostic assays using PCR and haemagglutination inhibition (HI) were carried out by the Veterinary Diagnostic Laboratory, Charles Sturt University, following published methods (Ypelaar et al., 1999; Khalesi et al., 2005). These tests have formed the basis for intensive monitoring of wild Orange-bellied Parrots for BFDV since 2000. PCR products from the Rep gene of BFDV, were purified using a commercial kit (QIAquick PCR Purification kit, Qiagen, Shanghai, China) and sequenced by a commercial laboratory (AGRF Ltd., Sydney, NSW, Australia) using a Sanger-based AB 3730xl unit (Applied Biosystems, Carlsbad, California, USA). High resolution melt curve analysis was also performed to identify variation in genetic sequences in the Rep (Sarker et al., 2014c) and capsid genes (Das unpublished, 2015), respectively. Whole genome sequencing was also performed using primer sets as described for previous BFDV genotypes from orange-bellied parrots (Sarker et al., 2014d). DNA sequences were trimmed and bidirectionally aligned using Sequencher v. 5.0.1 (Gene Codes Corp, Ann Arbor, Michigan, USA). Alignment and construction of the full genome sequence was carried out in a pairwise method with overlapping of between 265 and 394 nucleotides between the sequenced segments. Each aligned pair had no ambiguities and was used to generate a consensus sequence before aligning with the next sequence. Pairwise alignment used the inbuilt MAFFT v. 6.814b L-INS-i alignment algorithm in Geneious (Katoh et al., 2002). The full circovirus genome sequence constructed was aligned using the same MAFFT algorithm against all full BFDV genomes available on GenBank. Following alignment, NJ and ML phylogenetic analyses were carried out in Geneious. GTR with four categories was the optimal model of nucleotide substitution in jModelTest 2.1.3 (Darriba et al., 2012) and was used for ML while Hasegawa, Kishino and Yano (HKY) was used for the NJ method (Hasegawa et al., 2007). Each analysis was bootstrapped with 1,000 iterations and a majority consensus topology produced was produced for NJ.

## Results

All samples were negative for HI antibody (titre <1:20) but 20 of 23 birds tested positive by PCR.

### *High Resolution Melt (HRM) Analysis PCR*

HRM analysis was performed on positive results using both Cap and Rep genes with the greatest discrimination shown in the Cap gene. The results for this are shown in the Figure below. The initial results hinted that the outbreak was genetically different to the previous BFDV genotypes in the captive flock with individual amplicons clustering more closely with a sequence from a sulphur crested cockatoo obtained from NSW (blue line) than with a representative sequence from a captive Tasmanian Orange-bellied Parrot (0827-20213 shown as a purple line to the left) we sequenced in 2013.

### *BFDV DNA Sequencing Results*

DNA sequencing of the PCR positive samples yielded 13 entire genomes, all of which form a sister clade with the previous clade of BFDV from captive Orange-bellied Parrots at Taroona (outlined in blue below). Phylogenetic analysis of the new DNA sequences are shown in red text (only whole genomes are shown). In between these two clades is a single BFDV DNA sequence from a Bourke's parrot (KF688551) collected in Western Australia in 1996.

The topology shown in Figure 2 supports the divergence of the BFDV strain in the wild Orange-bellied Parrot from all other previously sequenced BFDV sequences. The topology shown in Figure 3 demonstrates remarkable diversity of infection in the 2015 outbreak in wild birds highlighting BFDV diversity within and between individual nestling family groups consistent with quasispecies theory.

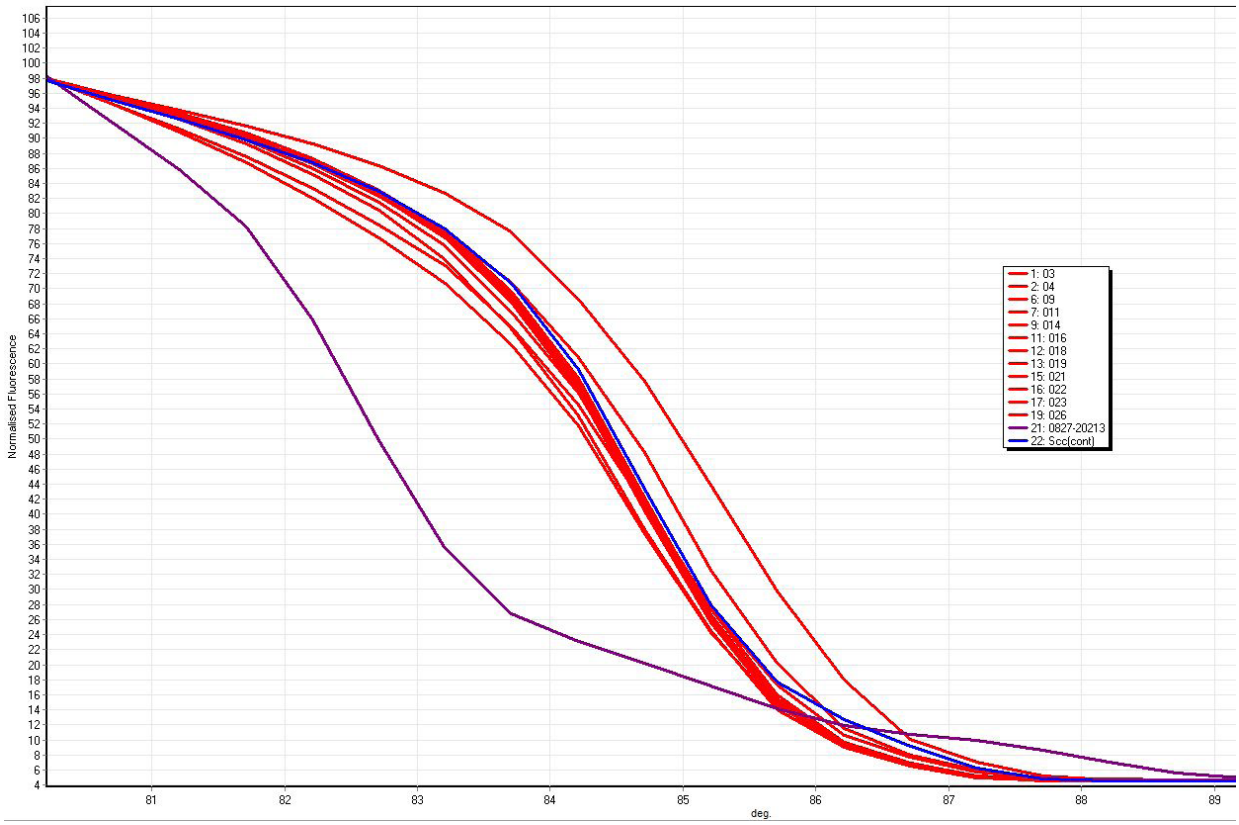


**Figure 1.** High Resolution Melt Analysis of BFDV using the capsid gene. Red curves show sequences from Orange-bellied Parrots sample in 2015 alongside a single wild Sulphur-crested Cockatoo (blue line) sampled in 1996.

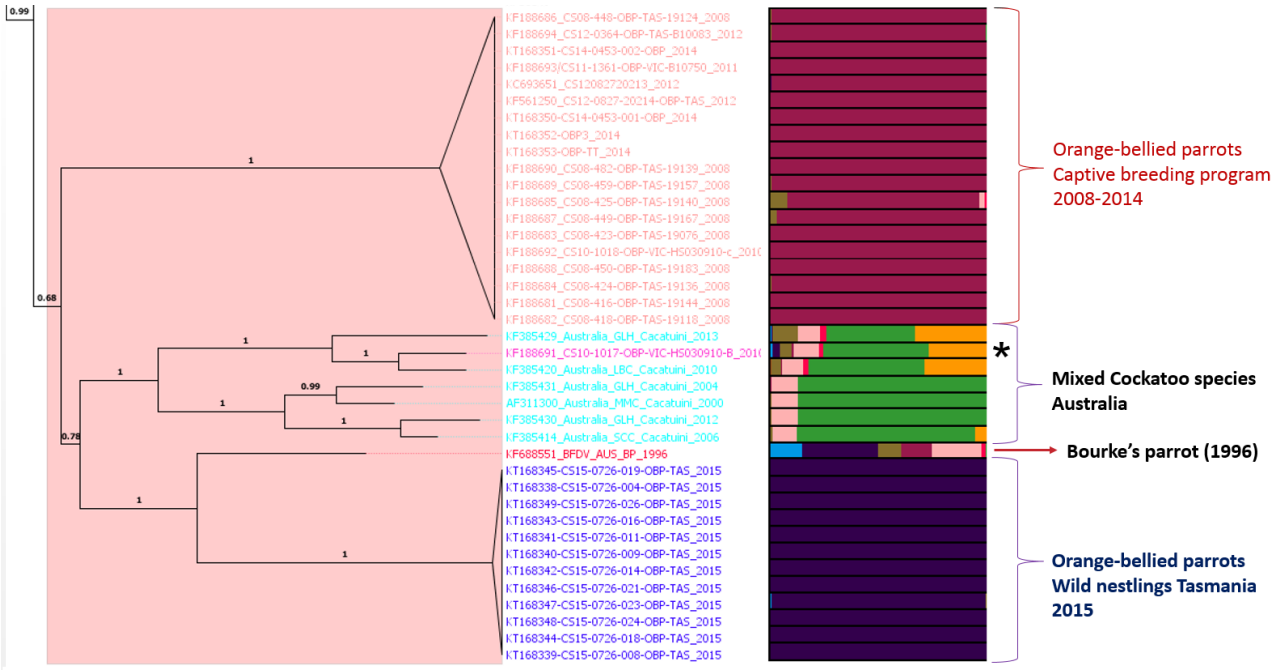


**Figure 2.** Bayesian phylogenetic analysis of BFDV sequences from wild Orange-bellied Parrots (2015) shown in blue text and their relationship to other closely related genotypes from captive Orange-bellied Parrots (Tasmanian flock) as well as mixed Australian species, mostly cockatoos but including one captive orange-bellied parrot in Victoria (asterisk).

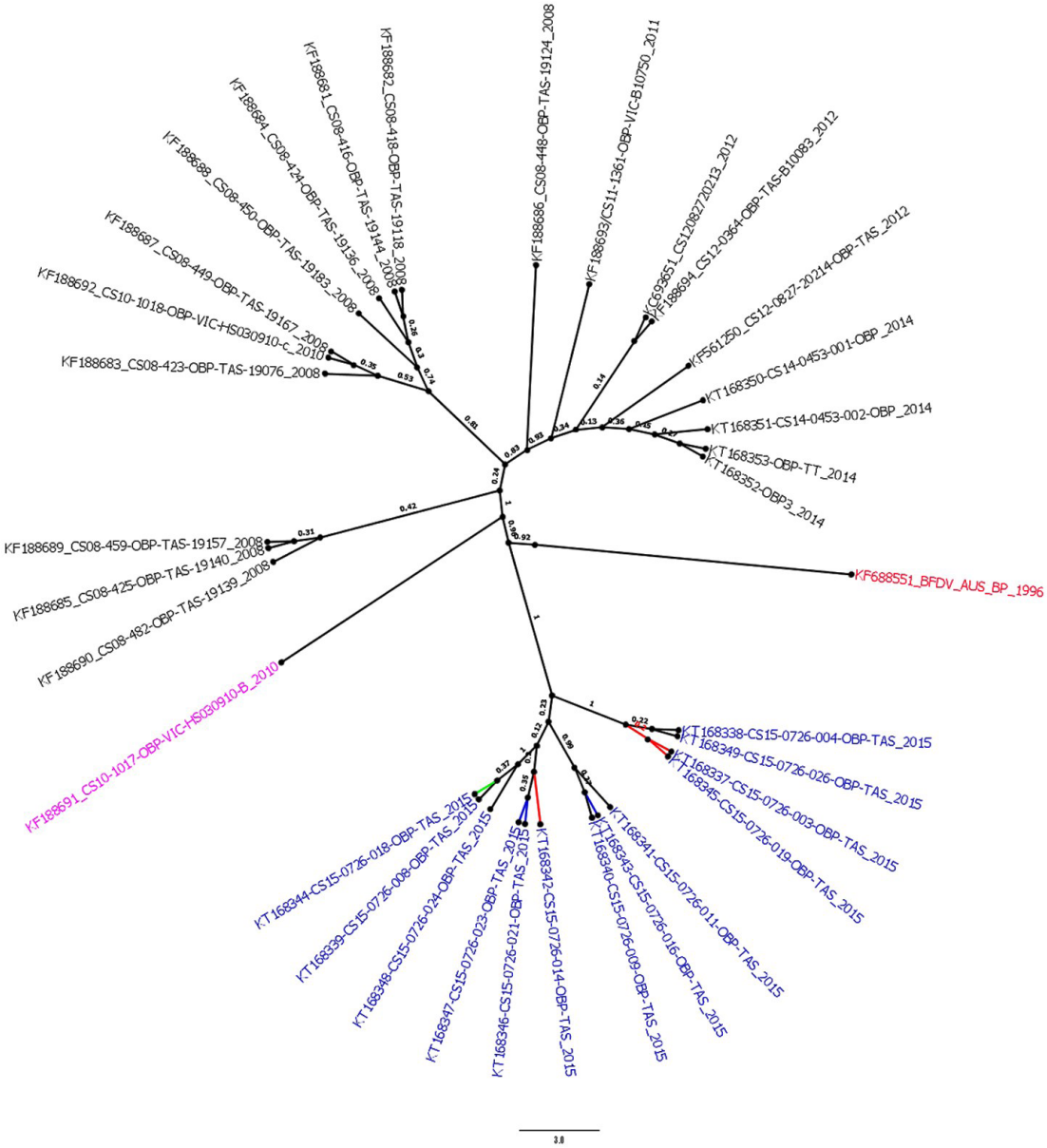


Figure 3 Relationship of BFDV from Orange-bellied Parrots from the wild in 2015 (blue text) compared to previously identified genotypes in captive Orange-bellied Parrots from Tasmania (black text), Victoria (pink text) and a wild Bourke's parrot (red text) collected in 1996. Different coloured lines represent genotypes taken from related nestlings in individual nest hollows.

## Discussion

This paper demonstrates a unique BFDV clade infecting wild nestling Orange-bellied Parrots during the 2014-15 breeding at Melaleuca in Tasmania, the last natural breeding population of the species. BFDV phylogenetic analysis provided strong branch support for the genetic relationship shown in Figure 2 with a relatively close genetic relationship to BFDV genotype KF688551 from a Bourke's parrot. The high degree of BFDV genetic diversity and admixture within the group sampled most likely reflects natural dispersal and transmission from other wild psittacine birds in the Melaleuca region. Within the wild BFDV subclade shown in Figure 3 similar viral sequences were obtained in different nestling groups which is best explained by a rich diversity of BFDV contamination in the environment rather than a single index case followed by rapid transmission.

The recent detection of two distinct lineages of BFDV in the remnant wild population of Orange-bellied Parrots, consisting of fewer than 50 birds, suggests a role for other parrot species as a reservoir for infection by spillover into this critically endangered species. The BFDV DNA sequence data strongly supports that the original outbreak in captive birds was caused by wild birds being brought into the captive breeding program after 2006 but before 2007-2008. After BFDV infection was confirmed in

several juvenile captive Orange-bellied Parrots in 2008 (Sarker et al., 2014d) a decision was made to test all birds in the captive breeding program and to sample the wild population in order to determine the source and extent of the infection. This revealed a high prevalence of infection in captive Tasmanian birds with 28.8% PCR positive and HI antibody titres ranging from 1:20 to 1:2,560 detected in 47 of 132 (35.6%) samples tested (Sarker et al., 2014d). In total 71/132 (53.8%) birds in the Tasmanian flock had laboratory evidence of current (PCR positive) or recent (HI positive) BFDV infection. Similarly, of 71 birds tested in the Victorian flock six were PCR positive and a further three birds were HI positive and most of these had a history that included translocation from Tasmania and/or South Australia. Of the 20 birds in the South Australian flock two were PCR positive. Efforts to control the disease included euthanasia of clinically diseased birds and segregation of clinically normal but PCR positive or HI positive birds and serial retesting.

The ability of circoviruses to persist in the environment for prolonged periods and their demonstrated ability to infect closely related hosts creates problems for managing this disease in wild flocks. The threshold susceptible population size required to endemically maintain BFDV in psittacine populations is unknown but, with less than 50 individuals, the wild population of Orange-bellied Parrots is almost certainly too small to maintain one, let alone different BFDV clades on its own (Raidal et al., 1993a, 1998; Swinton et al., 1998). In endemically infected flocks high antibody prevalence balanced by low disease prevalence reflects self-sustaining cycles of infection and immunologic stimulation which supports the development and maintenance of flock immunity (Raidal et al., 1993a). Flock seroprevalence is maintained due to high exposure to the pathogen at the expense of low losses. There is a trade-off between persistent infection and flock immunity.

The absence of detectable antibody in the blood of wild Orange-bellied Parrots is likely due to a combination of their young age as well as the presence of active infection. For more than two decades, over which the orange-bellied parrot has retained a very small remnant wild population, the species has probably lost most of it's endemic pathogens and it is likely that the flock behaves as a metapopulation for BFDV genotypes maintained in other species. The BFDV genotypes we detected currently infecting the species are likely to be the result of spillover through contact with other psittacine birds including cockatoos since the species is known to forage in mixed flocks.

High titres of BFDV are excreted in feces and feather material by infected birds and BFDV virions are capable of surviving in the environment of nest hollows for long periods (Raidal et al., 1993c). Like other circoviruses BFDV is resilient and can readily withstand temperatures of 80-85°C which may lead to the long-term contamination of nest hollows, perhaps for many years. Oral or cloacal transmission and environmental persistence of BFDV, as well as the predisposition of young birds to become infected, suggests a potentially significant role of shared nest hollows in facilitating spillover, allowing abundant parrot species to act as reservoirs for circovirus infection in very sparse or small populations of species such as the orange-bellied parrot. An analogous situation has been documented in Mauritius, where BFDV transmission occurs between invasive and abundant rose-ringed parakeets (*Psittacula krameri*) and the endemic, endangered Echo Parakeet (*Psittacula echo*) (Kundu et al., 2012).

The existence of reservoirs for BFDV in wild parrots throughout Tasmania is highly likely. The discovery of two divergent circovirus genotypes with different host affinities in two sibling swift parrot nestlings that had died acutely from PBFD in Tasmania demonstrates the likelihood of infection following shared nest hollow use by different parrot species (Khalesi et al., 2005; Sarker et al., 2013). The swift parrot is uniquely analogous to the orange-bellied parrot in that it is a small endangered migratory psittacine that breeds in nest hollows in Tasmania. The species has been observed reusing nest hollows when flowering conditions are favorable. It is not surprising that PBFD infects birds through shared nest hollows, for while a degree of host-specificity is seen in psittacine circoviruses considerable host-generalism is also observed in several lineages (Ortiz-Catedral et al., 2010; Varsani et al., 2010a, 2010b; Raidal et al., 2015; Sarker et al., 2015). Furthermore, the likely prolonged environmental persistence of circovirus virions (Raidal and Cross, 1994; Yilmaz and Kaleta, 2004) provides a mechanism by which transmission can occur in otherwise ecologically disconnected species. This is of particular significance in Australia, where 47 species of psittacine birds nest in tree hollows. At least four of these such as the Sulphur-crested Cockatoo (*Cacatua galerita*), Little Corella (Cacatua sanguine), Galah (*Eolophus rosei-*

*capillus*) and Rainbow Lorikeet (*Trichoglossus haematodus*) have expanded their range and population, and are known to occupy hollows used by other psittacine species. The Sulphur-crested Cockatoo was the first species in which PBFD was described and is known to maintain a high prevalence of antibody to BFDV in wild populations. The role of natural and artificial nest hollows in the transmission of BFDV and other viruses (Mackie et al., 2003) in wild Australian psittacine birds needs to be much better characterized.

While emerging infectious diseases of wildlife are receiving increasing attention as a potential cause of species extinction or endangerment (Daszak et al., 2000; Harvell 2004; Harvell et al., 2009) there remains limited evidence that infectious disease is a significant threatening process for the vast majority of the world's endangered species (perhaps with the exception of amphibians). This is in part due to the epidemiologic limitations on pathogen maintenance in small populations (De Castro and Bolker, 2005). There are however some circumstances under which, in theory at least, pathogens can lead to the extinction of their host. Key among these are firstly the introduction of a novel pathogen into a new host species; secondly the pre-epidemic host population is small; and the use of other abiotic or biotic reservoirs by the pathogen. It is possible that the emergence of PBFD in Orange-bellied Parrots represents a scenario incorporating all of these conditions. There is an urgent need to identify potential reservoir hosts for BFDV infection in southeastern Australia. This may be achieved through spatial and temporal analysis of full-genome phylogenies of PBFD in Orange-bellied Parrots and other Australian psittacine birds, especially those that share ecological space with this species. The re-emergence of BFDV in the wild orange-bellied parrot population is also a reminder that, even if infectious disease rarely is solely responsible for the extinction of a species, for populations that are critically endangered, emergent or introduced pathogens can present a significant risk. Indeed, Orange-bellied Parrots are also at risk from several other pathogens such as avian polyomavirus (Raidal et al., 1998) that are capable of causing catastrophically high mortality (Harris, 2006) and could result in the extinction of the few remaining birds through stochastic processes. It is crucial therefore that management of critically endangered species is highly attentive to the risks of infectious disease.

## References


Ashby, E., 1907. Parakeets moulting. Emu 6, 193-194.

Bassami, M.R., Berryman, D., Wilcox, G.E., Raidal, S.R., 1998. Psittacine beak and feather disease virus nucleotide sequence analysis and its relationship to porcine circovirus, plant circoviruses, and chicken anaemia virus. Virology 249, 453-459.

Bassami, M.R., Ypelaar, I., Berryman, D., Wilcox, G.E., Raidal, S.R., 2001. Genetic diversity of beak and feather disease virus detected in psittacine species in Australia. Virology 279, 392-400.

Brown, P.B., 1988. A captive breeding program for Orange-bellied Parrots. Australian Aviculture 42, 165-175.

Clout, M.N., Merton, D.V., 1998. Saving the Kakapo: the conservation of the world's most peculiar parrot. Bird Conservation International 8, 281-296.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9, 772.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287, 443-449.

De Castro, F., Bolker, B., 2005. Mechanisms of disease-induced extinction. Ecology Letters 8, 117-126.

Department of Sustainability Environment Water Population and Communities, 2013. Cyanoramphus cookii - Norfolk Island Parakeet, Tasman Parrot In Species Profile and Threats Database. Department of Sustainability, Environment, Water, Population and Communities, Canberra, Canberra.

Department of the Environment and Heritage, 2005. Threat Abatement Plan. Psittacine Beak and Feather Disease affecting endangered psittacine species. Department of the Environment and Heritage, Canberra, ACT 2601.

Drechsler, M., 1998. Spatial conservation management of the orange-bellied parrot *Neophema chrysogaster*. Biological Conservation 84, 283-292.

Ha, H.J., Anderson, I.L., Alley, M.R., Springett, B.P., Gartrell, B.D., 2007. The prevalence of beak and feather disease virus infection in wild populations of parrots and cockatoos in New Zealand. New Zealand Veterinary Journal 55, 235-238.

Harkins, G.W., Martin, D.P., Christoffels, A., Varsani, A., 2014. Towards inferring the global movement of beak and feather disease virus. Virology 450, 24-33.

Harris, J., 2006. Pathology, diagnostics, implications and strategies in a breeding colony of Orange-bellied Parrots (*Neophema chrysogaster*). In Australian Committee Association of Avian Veterinarians. ed. G. Cross, pp. 143-145. Association of Avian Veterinarians, Wellington, New Zealand.

Harvell, D., 2004. Ecology and evolution of host-pathogen interactions in nature. American Naturalist 164, S1-S5.

Harvell, D., Altizer, S., Cattadori, I.M., Harrington, L., Weil, E., 2009. Climate change and wildlife diseases: When does the host matter the most? Ecology 90, 912-920.

Hasegawa, H., Ichinohe, T., Tamura, S., Kurata, T., 2007. Development of a mucosal vaccine for influenza viruses: preparation for a potential influenza pandemic. Expert Review of Vaccines 6, 193-201.

Heath, L., Martin, D.P., Warburton, L., Perrin, M., Horsfield, W., Kingsley, C., Rybicki, E.P., Williamson, A.L., 2004. Evidence of unique genotypes of Beak and Feather disease virus in southern Africa. Journal of Virology 78, 9277-9284.

Jackson, B., Lorenzo, A., Theuerkauf, J., Barnaud, A., Duval, T., Guichard, P., Bloc, H., Baouma, A., Stainton, D., Kraberger, S., Murphy, S., Clark, N., Dillon, C., Knight, T., Varsani, A., 2014. Preliminary surveillance for beak and feather disease virus in wild parrots of New Caledonia: implications of a reservoir species for Ouvea Parakeets. Emu 114, 283-289.

Julian, L., Lorenzo, A., Chenuet, J.P., Bonzon, M., Marchal, C., Vignon, L., Collings, D.A., Walters, M., Jackson, B., Varsani, A., 2012. Evidence of multiple introductions of beak and feather disease virus into the Pacific islands of Nouvelle-Caledonie (New Caledonia). Journal of General Virology 93, 2466-2472.

Julian, L., Piasecki, T., Chrząstek, K., Walters, M., Muhire, B., Harkins, G.W., Martin, D.P., Varsani, A., 2013. Extensive recombination detected amongst Beak and feather disease virus isolates from breeding facilities in Poland. Journal of General Virology 94, 1086–1095.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30, 3059-3066.

Khalesi, B., Bonne, N., Stewart, M., Sharp, M., Raidal, S., 2005. A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from loriids. Journal of General Virology 86, 3039-3046.

Kundu, S., Faulkes, C.G., Greenwood, A.G., Jones, C.G., Kaiser, P., Lyne, O.D., Black, S.A., Chowrimootoo, A., Groombridge, J.J., 2012. Tracking viral evolution during a disease outbreak: the rapid and complete selective sweep of a circovirus in the endangered Echo parakeet. Journal of Virology 86, 5221-5229.

Layton, F., 1936. Runners or Hikers in budgerigars. The Central Queensland Herald (Rockhampton) 7, 11.

Leichhardt, L., 1846. Dr Leichhardts Lecture II. The Sydney Morning Herald 21, 2.

Mackie, J.T., Black, D., Prior, H., 2003. Enteritis associated with adenovirus-like particles in captive lorikeets. Australian Veterinary Journal 81, 293-295.

Massaro, M., Ortiz-Catedral, L., Julian, L., Galbraith, J.A., Kurenbach, B., Kearvell, J., Kemp, J., van Hal, J., Elkington, S., Taylor, G., Greene, T., van de Wetering, J., van de Wetering, M., Pryde, M., Dilks, P., Heber, S., Steeves, T.E., Walters, M., Shaw, S., Potter, J., Farrant, M., Brunton, D.H., Hauber, M., Jackson, B., Bell, P., Moorhouse, R., McInnes, K., Varsani, A., 2012. Molecular characterisation of beak and feather disease virus (BFDV) in New Zealand and its implications for managing an infectious disease. Archives of Virology 157, 1651-1663.

McOrist, S., Black, D.G., Pass, D.A., Scott, P.C., Marshall, J., 1984. Beak and feather dystrophy in wild sulphur-crested cockatoos (*Cacatua galerita*). Journal of Wildlife Diseases 20, 120-124.

Orange-bellied Parrot Recovery Team, 2013. National recovery plan for the Orange-bellied Parrot (*Neophema chrysogaster*).

Ortiz-Catedral, L., Kurenbach, B., Massaro, M., McInnes, K., Brunton, D.H., Hauber, M.E., Martin, D.P., Varsani, A., 2010. A new isolate of beak and feather disease virus from endemic wild red-fronted parakeets (*Cyanoramphus novaezelandiae*) in New Zealand. Archives of Virology 155, 613-620.

Ortiz-Catedral, L., McInnes, K., Hauber, M.E., Brunton, D.H., 2009. First report of beak and feather disease virus (BFDV) in wild Red-fronted Parakeets (Cyanoramphus novaezelandiae) in New Zealand. Emu 109, 244-247.

Pass, D.A., Perry, R.A., 1984. The pathology of psittacine beak and feather disease. Australian Veterinary Journal 61, 69-74.

Perry, R.A., Gill, J., Cross, G.M., 1991. Disorders of the avian integument. Veterinary Clinics of North America-Small Animal Practice 21, 1307-1327.

Peters, A., Patterson, E.I., Baker, B.G.B., Holdsworth, M., Sarker, S., Ghorashi, S.A., Raidal, S.R., 2014. Evidence of Psittacine Beak and Feather Disease Virus Spillover into Wild Critically Endangered Orange-bellied Parrots (*Neophema Chrysogaster*). Journal of Wildlife Diseases 50, 288-296.

Powell, A.M., 1903. Nude cockatoos. Emu 3, 55-56.

Raidal, S.R., Cross, G.M., 1994. The hemagglutination spectrum of psittacine beak and feather disease virus. Avian Pathology 23, 621-630.

Raidal, S.R., Cross, G.M., Tomaszewski, E., Graham, D.L., Phalen, D.N., 1998. A serologic survey for avian polyomavirus and Pacheco's disease virus in Australian cockatoos. Avian Pathology 27, 263-268.

Raidal, S.R., McElnea, C.L., Cross, G.M., 1993a. Seroprevalence of psittacine beak and feather disease in wild psittacine birds in New-South-Wales. Australian Veterinary Journal 70, 137-139.

Raidal, S.R., McElnea, C.L., Cross, G.M., 1993b. Seroprevalence of psittacine beak and feather disease in wild psittacine birds in New South Wales. Australian Veterinary Journal 70, 137-139.

Raidal, S.R., Sabine, M., Cross, G.M., 1993c. Laboratory diagnosis of psittacine beak and feather disease by hemagglutination and hemagglutination inhibition. Australian Veterinary Journal 70, 133-137.

Raidal, S.R., Sarker, S., Peters, A., 2015. A review of psittacine beak and feather disease and its impact on endangered species. Australian Veterinary Journal (In press).

Sarker, S., Forwood, J.K., Ghorashi, S.A., McLelland, D., Peters, A., Raidal, S.R., 2014a. Whole-Genome Sequence Characterization of a Beak and Feather Disease Virus in a Wild Regent Parrot (*Polytelis anthopeplus monarchoides*). Genome Announc 2.

Sarker, S., Forwood, J.K., Ghorashi, S.A., Peters, A., Raidal, S.R., 2015. Beak and feather disease virus genotypes in Australian parrots reveal flexible host-switching. Australian Veterinary Journal (In Press).

Sarker, S., Ghorashi, S.A., Forwood, J.K., Bent, S.J., Peters, A., Raidal, S.R., 2014b. Phylogeny of beak and feather disease virus in cockatoos demonstrates host generalism and multiple-variant infections within Psittaciformes. Virology 460-461, 72-82.

Sarker, S., Ghorashi, S.A., Forwood, J.K., Raidal, S.R., 2013. Whole-Genome Sequences of Two Beak and Feather Disease Viruses in the Endangered Swift Parrot (*Lathamus discolor*). Genome Announc 1.

Sarker, S., Ghorashi, S.A., Forwood, J.K., Raidal, S.R., 2014c. Rapid genotyping of beak and feather disease virus using high-resolution DNA melt curve analysis. Journal of Virological Methods 208, 47-55.

Sarker, S., Patterson, E.I., Peters, A., Baker, G.B., Forwood, J.K., Ghorashi, S.A., Holdsworth, M., Baker, R., Murray, N., Raidal, S.R., 2014d. Mutability dynamics of an emergent single stranded DNA virus in a naive host. PLoS ONE 9, e85370.

Stevenson, P., Yorkston, P., Greenwood, D., 1995. Draft Interim Plan. Green Parrot Recovery Program 1995-96, ed. A.N.C.A. Norfolk Island Conservancy. Australian Nature Conservation Agency. Commonwealth of Australia. (1993-1998) Canberra.

Swinton, J., Harwood, J., Grenfell, B.T., Gilligan, C.A., 1998. Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* meta-

populations. Journal of Animal Ecology 67, 54-68.

Varsani, A., de Villiers, G.K., Regnard, G.L., Bragg, R.R., Kondiah, K., Hitzeroth, II, Rybicki, E.P., 2010a. A unique isolate of beak and feather disease virus isolated from budgerigars (*Melopsittacus undulatus*) in South Africa. Archives of Virology 155, 435-439.

Varsani, A., Regnard, G.L., Bragg, R., Hitzeroth, II, Rybicki, E.P., 2010b. Global genetic diversity and geographical and host-species distribution of beak and feather disease virus isolates. Journal of General Virology 92, 752-767.

Yilmaz, A., Kaleta, E.F., 2004. Disinfectant tests at 20 and 10 degrees C to evaluate of virucidal activity against circoviruses. Deutsche Tierarztliche Wochenschrift 111, 248-251.

Ypelaar, I., Bassami, M.R., Wilcox, G.E., Raidal, S.R., 1999. A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. Veterinary Microbiology 68, 141-148.