PSITTACINE BEAK AND FEATHER DISEASE IN ORANGE-BELLIED PARROTS (*Neophema chrysogaster*)

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INTRODUCTION

Psittacine Beak and Feather Disease (PBFD) was listed in April 2001 as a key threatening process under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) and it is a disease threat to the critically endangered orange-bellied parrot (*Neophema chrysogaster*) and at least 25 other Australasian bird species or subspecies that are listed as either endangered or vulnerable under provisions of the EPBC Act (Table 1). PBFD causes either a chronic debilitating feather disease in adult birds (Pass and Perry, 1985; Ritchie et al., 1989b; Rahaus and Wolff, 2003; Albertyn et al., 2004) or a severe, acute disease syndrome in nestling parrots (Raidal and Cross, 1995; Schoemaker et al., 2000). The causative agent, BFDV, is a circovirus with a small single stranded DNA genome of only 1.7-2.0 kb (Ritchie et al., 1989; Bassami et al., 2001) and is now considered to have a worldwide distribution (McOrist et al., 1984; Pass and Perry, 1985; Ritchie et al., 1989a; Kock et al., 1993; Kiatipattanasakul-Banlunara et al., 2002; Rahaus and Wolff, 2003; Albertyn et al., 2004; Hsu et al., 2006). Surveys using PCR-based assay methods have found prevalence rates of between 8% (Bert et al., 2005) and 39% (Rahaus and Wolff, 2003) and the reported seroprevalence varies between 16% and 62% (Raidal and Cross, 1994; Khalesi et al., 2005) among captive flocks and between 41% and 94% in wild flocks (Raidal et al., 1993).

Infectious diseases are often mooted as possible threats to endangered bird species but there is very little research into the ecological impact that such diseases actually have on threatened populations. PBFD was recognised as a disease of concern in the *National Recovery Plan for the Orange bellied Parrot* (see URL-1) because it was detected in wild birds in 1993 (Brown 1988). However, it was believed to have been eradicated from the captive population since 1991 through the relocation of the Hobart facility to a warmer, more sheltered site at Taroona. Whilst there has been some *ad hoc* monitoring of the disease status of captive populations in Hobart, Melbourne and Adelaide there have been no published records to document this. The estimated current population of orange bellied parrots is now fewer than 200 birds and the species range coincides with a number of recently constructed wind power generation facilities (wind farms) which has attracted significant media and political attention and, while recent reports have implicated wind farms as a significant threat to the species (Cumulative risks for threatened and migratory species, Biosis Research Pty Ltd), infectious diseases are probably a much more serious immediate threat to the survival of this species. This is highlighted by a recent epidemic of mortalities in progeny birds for which no aetiological cause has been identified (Harris, 2006). Since this outbreak of mortalities there has been a heightened surveillance of the captive population. In the summer breeding season of 2007-08 several juvenile birds at Taroona developed clinical signs of PBFD which was confirmed by laboratory testing. A decision was made to test all birds in the captive breeding programme and to sample wild populations of OBP (and associated BWPs) to determine the extent of the infection. The results of this testing are presented below.

LABORATORY TESTING

PCR, as well as haemagglutination (HA) and haemagglutination-inhibition (HI) assays were performed on blood and feather samples. Blood was collected by venepuncture of the jugular, or brachial vein and spotted onto filter paper (Whatmann, No. 3), then allowed to air dry at room temperature as described by Riddoch et al. (1996). Feathers were plucked and placed into clean 1.5 mL microcentrifuge tubes. PCR and HI was performed on blood and feathers were used for HA testing.

Polymerase Chain Reaction (PCR)

Viral DNA was extracted from feathers and PCR was carried out using methods similar to those described by Ypelaar et al. (1999). Reactions were carried out in an Eppendorf Mastercycler Gradient thermocycler (Eppendorf). PCR products were visualised on a 1% agarose gel with the addition of 0.001% ethidium bromide, run at 90V for 30 minutes.

Haemagglutination (HA) and Haemagglutination Inhibition (HI) Assays

HA and HI assays were carried out as described by Raidal *et al.*, (1993b). Antigen purified from the feathers of a cockatoo with PBFD was used in the HI assay. Plasma, serum or dried blood spots on filter paper were used for testing from the birds.

RESULTS

Samples from Tasmania (n=132) included captive-bred juveniles and adults, and wild birds from several locations (Melaleuca and Birchs inlet). Of these 35 were PCR positive on blood samples and a further 3 birds were positive on feather samples (28.8% PCR positive). HI antibody titres ranging from 1:20 to 1:2,560 were detected in 47 of 132 (35.6%) samples tested. In total 71/132 (53.8%) Tasmanian birds had laboratory evidence of current (PCR positive) or previous (HI positive) BFDV

infection. Three birds that were PCR positive also had clinical signs of PBFD and high feather HA results (titres ranging from 1:80 to 1:10,240).

Of 71 birds tested at Healesville Sanctuary six were PCR positive and a further 3 birds were HI positive. Most of these birds had a history that included translocation either from Taroona and or Adelaide. Efforts to control the disease in both flocks include euthanasia of clinically diseased birds and segregation of clinically normal but PCR positive or HI positive birds.

Of 20 birds tested at Adelaide Zoo two were PCR positive (another laboratory provided these results).

DISCUSSION

The results indicate that there is a high prevalence of BFDV infection in captive orange bellied parrots in the Tasmanian population. DNA sequencing of PCR positive amplicons has shown a high degree of similarity between individual isolates, effectively all have been identical.

It is not known how susceptible orange-bellied parrots are to isolates or strains of BFDV derived from other psittacine bird species such as from rosellas or lorikeets. Nevertheless, PBFD has been confirmed in more than 60 psittacine species and it is highly likely that all are susceptible (Pass and Perry, 1985; Ritchie *et al.*, 1989; Rahaus and Wolff, 2003; Albertyn *et al.*, 2004). Surveys have been carried out in both wild and captive psittacine populations and reported virus prevalence rates vary between 10-94%, depending on the method of detection (McOrist *et al.*, 1984; Raidal *et al.*, 1993a; Rahaus and Wolff, 2003; Khalesi *et al.*, 2005). In endemically infected flocks an expected seroprevalence of 30-40% would be a conservative estimate of the percentage of birds that recovery from infection and mount an appropriate immune response (Raidal *et al.,* 1993a; Raidal and Cross 1994b; Khalesi *et al.,* 2005).

In the early stages of infection naïve flocks are likely to have a high rate of birds with active infection (PCR positive) with or without the development of detectable BFDV excretion (by HA) from feathers and faeces. A PCR prevalence of almost 30% is a relatively high attack rate and similar to results obtained for lovebirds (Khalesi *et al.*, 2005). However, it is unknown how orange bellied parrots or other *Neophema* species react to BFDV infection on a flock basis.

Very little is known about the population size that is needed to maintain disease agents in wild populations of animals (Swinton *et al.,* 1998; Lloyd-Smith 2005). For example, persistence thresholds have been estimated for phocine distemper virus in harbour seals (Swinton *et al.,* 1998). There is a minimum population size that exists whereby pathogens cannot be maintained either because the number of susceptible or actively shedding animals is too small to maintain sufficient disease transmission. Factors such as the environmental and antigenic stability of the agent; the main methods of transmission; the age window of susceptibility of the host; and the persistence of protective antibody, all influence the population size threshold that can maintain on-going infection. Host population thresholds for the invasion or persistence of infectious disease are core concepts of disease ecology and underlie disease control policies based on culling and vaccination and can significantly assist the management of critically endangered species populations (Lloyd-Smith *et al.,* 2005).

One of the options for endangered species conservation programs is to create insurance populations to minimise the detrimental effects of a catastrophic infectious disease outbreak. This was a major reason for establishing flocks at Healesville Sanctuary and Adelaide Zoo. The DNA sequencing results

indicate that there is evidence of recent BFDV infection in all 3 captive breeding populations which may have serious consequences for the viability of this approach. Culling is a controversial option for a species that is critically endangered. Another option may be to isolate known infected and diseased birds and use novel techniques to recuperate breeding birds in order to preserve their genes. For example, diseased male birds may pose a low risk of viral transmission to immune female birds and their progeny. Whereas, diseased female birds might be able to produce BFDV-free eggs that could be incubated and raised by foster birds. Such knowledge might not yet be vital for the survival of the species, but one could argue that the time could soon be approaching when artificial and assisted breeding is essential.

One of the difficulties for controlling BFDV in a species with a small population size is that it is highly likely that cross-species transmission of infection occurs. This has been demonstrated to have occurred in the nest site of wild swift parrots on Bruny Island, Tasmania (Khalesi *et al.,* 2005). Furthermore, phylogenetic tree analysis demonstrated that swift parrot nestlings from the same clutch could be infected with lorikeet derived BFDV as well as parrot derived BFDV genotypes. Thus it is highly likely that the more common psittacine bird species act as reservoirs of infection for endangered species. Such a situation creates an imperative for all captive bred orange bellied parrots to be housed in covered aviaries that cannot be accidentally contaminated by free-flying wild birds as well as the correct implementation of disinfectant and decontamination procedures to minimise viral loads.

It should be noted that the Commonwealth of Australia does have an existing Threat Abatement Plan (TAP) for *Psittacine Beak and Feather Disease Affecting Endangered Psittacine Species* (see URL-2). In 2005 this was ratified by the Minister for the Environment and Heritage, under section 270B(2) of the Environment Protection and Biodiversity Conservation Act 1999. The TAP contains a list of key actions and research objectives that have yet to be empowered or achieved. Perhaps the AAVAC needs to be more involved in agitating for the critical review and or implementation of such policies.

The pathogenesis and expression of viral disease in individual birds and flocks can be influenced by stress and the general health status of the birds. Hygiene control measures that minimise viral loads in key fomites such as nesting hollows and feeding utensils can also greatly reduce the transmission and minimise infectivity rates. All nest boxes for the Tasmanian flock are destroyed annually and Vircon is used to disinfect equipment. The control of other diseases that have the potential to impair the immune system is also important. Non-specific immunostimulants that kindle the innate immune response have been shown to produce some protective benefit on a flock basis but are not as effective as vaccination to stimulate a long lasting specific adaptive immune response. Vaccines are not a substitute for good hygiene and management practices for the control of any viral disease. Nevertheless, an effective vaccine is likely to be the single most important tool for minimising the threat of PBFD to the recovery program of the orange bellied parrot.

List of Extinct, Threatened and Near Threatened Australian Psittacine Birds 2008 (see URL-3)

Species	Common Name	Status
Cacatua leadbeateri leadbeateri	Major Mitchell's Cockatoo (Eastern)	Near Threatened
Cacatua pastinator pastinator	Muir's Corella	Endangered
Calyptorhynchus banksii graptogyne	Red-tailed Black-Cockatoo (SE)	Endangered
Calyptorhynchus banksii naso	Red-tailed Black-Cockatoo (SW)	Near Threatened
Calyptorhynchus baudinii	Baudin's Black-Cockatoo	Near Threatened
Calyptorhynchus lathami halmaturinus	Glossy Black-Cockatoo (Kangaroo Is)	Endangered
Calyptorhynchus lathami lathami	Glossy Black-Cockatoo (eastern)	Near Threatened
Calyptorhynchus latirostris	Carnaby's Black-Cockatoo	Endangered
Cyanoramphus cookii	Norfolk Island Green Parrot	Endangered
Cyanoramphus n. subflavescens	Red-crowned Parakeet (Lord Howe Is)	Extinct
Cyanoramphus novaezelandiae erythrotis	Red-crowned Parakeet (Macquarie Is)	Extinct
Cyclopsitta diophthalma coxeni	Coxens Fig-Parrot	Critically Endangered
Eclectus roratus macgillivrayi	Eclectus Parrot (Cape York Peninsula)	Near Threatened
Eclectus roratus polychloros	Eclectus Parrot (Torres Strait)	Near Threatened
Geoffroyus geoffroyi maclennani	Red-cheeked Parrot (Australian)	Near Threatened
Lathamus discolor	Swift Parrot	Endangered
Neophema chrysogaster	Orange-bellied Parrot	Critically Endangered
Neophema pulchella	Turquoise Parrot	Near Threatened
Nestor productus	Norfolk Island Kaka	Extinct
Pezoporus occidentalis	Night Parrot	Critically Endangered
Pezoporus wallicus flaviventris	Ground Parrot (western)	Endangered
Pezoporus wallicus wallicus	Ground Parrot (eastern)	Vulnerable
Platycercus caledonicus brownii	Green Rosella (King Island)	Vulnerable
Platycercus eximius diemenensis	Eastern Rosella (Tasmanian)	Near Threatened
Platycercus icterotis xanthogenys	Western Rosella (wheatbelt)	Near Threatened
Polytelis alexandrae	Princess Parrot	Near Threatened
Polytelis anthopeplus monarchoides	Regent Parrot (Eastern)	Endangered
Polytelis swainsonii	Superb Parrot	Vulnerable
Probosciger aterrimus macgillivrayi	Palm Cockatoo Southern	Near Threatened
Psephotus chrysopterygius	Golden-shouldered Parrot	Endangered
Psephotus pulcherrimus	Paradise Parrot	Extinct

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