

# A survey of wild Australian psittacine birds for evidence of infection by avian polyomavirus and Pacheco's disease virus

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## Introduction

Avian polyomavirus (APV) disease is commonly diagnosed in captive psittacine birds in Australia, mostly in captive-bred nestling budgerigars (*Melopsittacus undulatus*) and lovebirds (*Agapornis* sp.) (Pass 1985, 1987; Reece *et al.*, 1992). Evidence of APV infection and disease in wild cockatoos has been limited to the detection of polyomavirus-like particles in the faeces of galahs (*Eolophus roseicapillus*) in the Perth Metropolitan area (D.A. Pass and S.L. Plant *pers comm* 1996).

Pacheco's disease (PD) is an acute fatal disease of psittacine birds caused by psittacid herpesvirus 1 (PsiHV1). Outside of Australia, PsiHV1 has been isolated from captive psittacine birds native to Africa, the Americas, and Australasia. The virus probably originated in wild birds from South America, as it was first recognized in Brazil (Pacheco and Bier, 1930). PD has never been confirmed in wild or captive psittacine birds within Australia. Recently respiratory diseases with herpesvirus-like lesions have been reported in captive Bourke parrots (*Neophema bourkii*) and Indian ringneck parrots (*Psittacula krameri manillensis*) in Australia (Charles 1995; Raidal *et al.*, 1995).

The primary purpose of the investigations presented in this report was to survey 411 wild and captive Australian psittacine birds for serological evidence of APV and PsiHV1 infection.

## Results

### APV serology for wild birds

Neutralizing antibody to APV was detected in 96 (64.4%) blood samples from 149 SCC

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trapped in Sydney and Yeoval. In these flocks, the prevalence of antibody positive SCC ranged from 56% to 87.5%. Antibody titres ranged from 1:32 to 1:2048 with a median value of 1:64. Antibody was not found in the adult or nestling galahs from Yeoval (n=78). Only 2 of the 17 LBC (12%) trapped in the Sydney area had neutralizing antibody. The galahs (n=18), SBC (n=6), and gang gang cockatoos (n=4) from the Sydney area were seronegative.

The SCC nestlings (n=14) from Yass were seronegative at the first sampling. One of six nestlings seroconverted by the time of the second sampling (4 weeks after PsCV-vaccination and challenge) and an additional 4 birds were seropositive after 3 months. Antibody titres ranged from 1:512 to 1:2048. Signs of illness were not detected in these birds during or after the sampling period.

Neutralizing antibody to APV was not detected in samples (n=16) obtained from birds at MWNP nor in samples from orange-bellied parrots (n=36) in captive breeding programmes.

#### **APV serology for captive psittacine birds**

Neutralizing antibody was detected in 16 of 44 birds (36.4%) of the birds from Flock 1 (Table 1). Of these birds at least 2 SCC and 1 RTBC were tested positive more than once during the sampling period. Neutralizing antibody to APV was not detected in Flock 2 and Flock 3.

#### **Pacheco's disease serology**

All 12 vaccinated macaws had neutralizing antibody (titres >1:16) to PsiHV1. Serum from the 4 hand-raised blue and gold macaws exhibited no neutralizing activity. All 411 Australian birds were negative for neutralizing antibody to PsiHV1.

**Table 1.** Species and APV seropositive birds in Flock 1, a mixed flock of cockatoos and parrots in New South Wales sampled over a 14 month period.

Species*	Number seropositive / number tested
Sulphur-crested cockatoo ( <i>Cacatua galerita</i> )	7 / 11
Major Mitchell's cockatoo ( <i>Cacatua leadbeateri</i> )	3 / 4
Gang gang cockatoo ( <i>Callacocephalon fimbriatum</i> )	1 / 6
Galah ( <i>Eolophus roseicapillus</i> )	1 / 7
White-tailed black cockatoo ( <i>Calyptorhynchus latirostris</i> )	1 / 3
	1 / 2
Red-tailed black cockatoo ( <i>Calyptorhynchus magnificus</i> )	1 / 5
Rainbow lorikeet ( <i>Trichoglossus haematodus</i> )	0 / 1
Varied lorikeet ( <i>Trichoglossus versicolor</i> )	1 / 1
Scaley-breasted lorikeet ( <i>Trichoglossus chlorolepidotus</i> )	

<sup>a</sup> At least one sulphur crested cockatoo and one red-tailed black cockatoo were tested positive more than once during the sampling period. An additional bird was sampled and found to be seronegative, the species of this bird was not recorded at the time of collection.

## Discussion

The high seroprevalence and at times high APV-neutralizing antibody titres detected in wild SCCs indicates that SCCs in NSW are enzootically infected with APV. Neither systemic nor integumentary disease attributable to APV has been confirmed in the large number of nestling and fledgling cockatoos trapped for the pet bird market annually. However, studies in the United States of America show that APV is highly infectious to psittacine birds of all ages, but APV disease is predominantly confined to nestlings of a few parrot species. Although cockatoos appear to be readily susceptible to APV infection, disease in nestling and adult birds is extremely rare (Wainwright et al., 1987; Phalen et al., 1997a) and these rare cases of APV-disease may only occur in cockatoos concurrently infected with and immunosuppressed by PsCV (Latimer et al., 1993, 1996; Phalen et al., 1997b).

While antibodies were not present in SCC nestlings taken from the wild in Yass, 5 of 6 PsCV-

vaccinated birds seroconverted while in captivity. How the virus was introduced to these birds is not known. It is possible however, that one of the wild caught birds was incubating APV at the time of capture but had not yet seroconverted then subsequently became a source of virus for other chicks in the group. The inactivated antigen and PsCV inoculum used to vaccinate and challenge the birds may have been contaminated with APV. However, other birds including SCC, galahs and other cockatoos that have received similar treatment failed to seroconvert (S.R. Raidal, G.M. Cross and D.N. Phalen unpublished observation).

The absence of anti-APV antibody titres in the wild-caught galahs and short-billed correllas and the low prevalence of antibody titres in LBC remains unexplained. It was similarly unexpected that neutralizing antibody was found in only a single galah in Flock 1. In captive parrots in the United States, once APV is introduced into a collection, it rapidly disseminates (Phalen *et al.*, 1997a). Galahs and SCC form large mixed flocks and cross-transmission with PsCV between these birds appears to occur (Raidal *et al.* 1993b). It was thought that APV would spread between these species as well. Two possible explanations may account for the findings. Firstly, it has been shown that anti-APV antibody titres may only be maintained for 6 months or less in certain parrot species, so these birds may have been infected previously, but subsequently had become seronegative (Wainright, *et al.*, 1987; Phalen *et al.*, 1997a). Another explanation would be that the antibodies detected in wild SCC were due to infection with a relatively species-specific variant of this virus.

Recent experiments indicate that at least one naturally occurring variant of APV with some host specificity exists. This European APV isolate did not grow in chicken embryo fibroblasts whereas two other European APV isolates did so readily. The observed *in vitro* growth restriction was documented to be the result of a single point mutation in the overlapping coding sequence for the APV's VP2/VP3 structural proteins (Stoll *et al.*, 1994). Additional sequence analysis of 17 North American APV isolates has documented that a small segment of the VP2/3 open reading frame surrounding this mutation is unusually prone to nonsynonymous mutations. It has been suggested that these mutations may enhance virus packaging in one psittacine species, but not necessarily in another (Phalen, *et al.*, 1997c). In avicultural collections containing numerous species of parrots, an APV mutation resulting in a reduced host range would be of little advantage to the virus. This may explain why these VP2/VP3 mutations were generally found only at the terminal branches of the APV-phylogenetic tree. In contrast, a similar mutation causing improved replication efficiency in single species that congregate in large flocks with other members of its species, such as the SCC, may be highly advantageous.

In the present study antibodies to APV were not detected in captive Flocks 2 and 3 in Western Australia. It is noteworthy that these flocks did not contain SCC. The natural range of SCC does not extend to this part of Australia and SCC are not commonly available through pet shops because the Western Australian government has banned importation of this species from the eastern states.

Our data do not explain previous observations of APV-like particles in wild galahs trapped in the Perth area (S Plant and D Pass *pers comm*). The galahs that these workers examined could have been escapee birds that had been trapped in Victoria and transported to the pet market in Perth. Serologic surveys of galahs and other cockatoos from this area will be needed to better understand these observations.

APV appeared suddenly in the early 1980's in North America (Bernier *et al.*, 1981; Davis *et al.*, 1981) and has since been documented in captive birds from around the world. In which bird

population or populations this virus originated has yet to be determined. This is the second study to report anti-APV antibodies in wild populations of parrots. In the first report, anti-APV activity was found in serum samples from 5 of 10 dusky-headed parakeets (*Aratinga weddellii*) trapped in southeastern Peru (Gilardi et al., 1995). In this study, sera were screened for APV antibody using a complement fixation (CF) assay done at the Texas Veterinary Medical Diagnostic Laboratory and by the same VN employed in this report. Not all samples, however, were examined by both methods and when both methods were used results were not always consistent. More importantly, the serum was not heat inactivated and the VN titres were  $\leq 1:32$  (D.N. Phalen *personal communication*). Subsequent work has shown that such low levels of neutralizing activity susceptible to heat inactivation may be found in *Aratinga* sp. (D.N. Phalen, unpublished observation), suggesting that the low neutralizing activity found in the dusky-headed parakeets was not antibody. Additionally, a study comparing the CF and VN using carefully defined positive and negative serum samples, showed only a correlation of 0.60 between the CF and VN (D.N. Phalen, unpublished observation). As the VN assay has been proven to be a sensitive and specific measure of anti-APV antibody in the hands of several authors (Wainwright et al., 1987; Ritchie, et al., 1994), we must question whether this particular CF actually measures anti-APV antibodies. In contrast to the dusky-headed parrot study, the serum and plasma examined in the present investigation were heat inactivated and neutralizing activity titres as high as 1:2024 were found. Considering all of these factors, we speculate that wild Australian birds, possibly the SCC, may represent the original source of APV.

Herpesvirus diseases, in general, appear to be rare in psittacine birds in Australia. Only recently have respiratory diseases been reported in captive Bourke's parrots (*Neophema bourkii*) and Indian ringneck parrots (*Psittacula krameri manillensis*) in Australia (Charles 1996; Raidel *et al.*, 1995). PD and similar diseases have not been reported in Australia. Although many PDV isolates are at least partially serologically cross-reactive (Gravendyck *et al.*, 1996), we cannot completely rule out that non-cross-reactive PDV serotypes may have been present in these birds. The relationship of PDV to the herpesvirus-like respiratory disease, recently described in Australia, is not known. Similar lesions in Amazon parrots and Bourke's parrots have been described in Europe and in parakeets (*Psittacula krameri manillensis*), Amazons (*Amazona aestiva aestiva*) and cockatiels (*Nymphicus hollandicus*) in Japan (Tsai et al., 1993). The European herpesvirus has been suggested to a variant of the poultry laryngotracheitis virus (Helfer, 1980; Winterroll, 1977). The Japanese isolates have not been characterised.

In conclusion, we found serological evidence of APV infection to be widespread and common in SCC's from NSW. The presumed APV infecting wild SCCs appears to be highly host adapted, as it has not been correlated with overt APV-disease. We also suggest, that the APV identified in SCC may represent the original APV. In contrast, we found no serological evidence for PsiHV1 in Australia and suggest that Australia may be free of this disease.

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