Avian papovaviruses

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Papovaviruses are small, naked, icosahedral, double stranded, circular DNA viruses which replicate in the nuclei of their host cells. The group derives its name from the initials of the three main types: papilloma (55 nm in diameter), polyoma (45 nm in diameter) and vacuolating virus (Thomas, 1988). Vacuolating virus has not been reported in avian species and will not be further discussed in this paper.

1. POLYOMAVIRUSES

Polyomavirus is a genus in the family Papovaviridae which is generally associated with a variety of tumour cells of laboratory animals. These viruses are nearly always integrated into host cell DNA and may be transmitted by contact with saliva, faeces or urine (Thomas, 1988). In birds, polyomavirus has been associated with systemic disease but not yet with tumours. Polyoma and polyoma-like viruses have been associated with disease in captive and wild birds in Australia and in captive birds overseas.

1.1 Polyomavirus in budgerigars

1.1.1 Budgerigar Fledgling Disease

Polyomavirus was first reported as the cause of deaths and feather abnormalities in juvenile budgerigars (*Melopsittacus undulatus*) in the United States (Bernier *et al*., 1981; Davis *et al*., 1981). Newly hatched birds were most often affected, developing normally for the first two weeks or longer, then suddenly dying. Clinical signs included abdominal distension, blue discolouration of the skin (subcutaneous haemorrhage), retarded growth and, in some cases, abnormal growth of feathers. In survivors, feathers failed to grow and the birds were unable to fly. Gross necropsy findings included hydropericardium, enlarged heart and liver with multiple pinpoint white spots or large yellow foci, pale congested kidneys and occasionally ascites. Electron micrographs showed viral particles 42 to 49 nm in diameter in the nuclei of epithelial cells of the renal tubules. This syndrome was termed "budgerigar fledgling disease" (BFD). A later report described tremors in some nestling budgerigars with BFD (Mathey and Cho, 1984).

A second clinical syndrome was also associated with this virus in budgies.

Newly hatched birds showed feather abnormalities and older fledglings lost tail and flight feathers. The signs were very similar to those described as "French moult" and there was speculation that this syndrome represented a more delayed, milder form of the acute disease affecting newly hatched budgies.

Bernier *et al*. (1981) reported large, pleomorphic, intranuclear inclusion bodies with a clear centre and containing very small, slightly basophilic granules in many organs of affected budgies. These inclusions were demonstrated in birds as young as one day old, suggesting that the disease could be egg transmitted. Bernier *et al*. (1981) were able to transmit the disease by intranasal inoculation with a bacteria-free supernatant of a homogenate prepared from skin tissue of naturally infected birds. Bozeman *et al*. (1981) isolated BFD virus in budgerigar embryo fibroblasts. Cytopathic effect in fibroblasts was characterised by a swollen nucleus followed by rounding and detachment of the affected cell from the monolayer. The isolate did not haemagglutinate erythrocytes. A fluorescent-antibody virus neutralisation (FAVN) test specific for BFD virus was later developed by Wainright *et al*. (1987).

1.1.2 **Reports of BFD from regions other than North America**

BFD has been reported from the United States (Davis, 1981, 1983a, 1983b), Canada (Bernier *et al*., 1981), Japan (Hirai *et al*., 1984), Italy (Pascucci *et al*., 1983), Hungary (Sztojkov *et al*., 1985), Britain (Randall *et al*., 1987) and Germany (Krautwald *et al*., 1985).

In the first British report (Randall *et al*., 1987) mortality varied between 20% and 50%, signs were first observed at 5 to 7 days of age, most birds had darkening of the normal skin colour followed by death in 2 to 10 days. Occasional survivors had abnormal plumage. There were few changes on gross autopsy but histologically many large basophilic intranuclear inclusions were present in multifocal degenerative lesions throughout the epidermis, kidney, heart, brain and other tissues. Ultrastructural studies demonstrated numerous viral particles with polyomavirus-like morphology.

Hirai *et al*. (1984) described a condition where there was abnormal development of flight and tail feathers in 3-4 week old budgerigars. No gross organ changes were evident at autopsy but microscopic examination of the kidneys revealed markedly enlarged nuclei with opaque inclusions most prominent in the epithelial cells of the renal tubules. Similar inclusions were also present in the feather follicle sheath cells of the feather follicles.

In speaking to budgie breeders at club meetings in Melbourne in the 1980's it was apparent that a disease similar BFD had been occurring in local budgies for many years. Breeders described underdeveloped chicks that failed to grow, "looked like little red marbles" and died within one to three weeks of hatching. A number of different breeders described such chicks as occurring from time

to time in their aviaries. The birds were not taken to veterinarians because the owners felt that the value of the birds did not warrant the expense of veterinary consultation and losses tended to be sporadic. Following these discussions several affected chicks were submitted to the Veterinary Research Institute of Victoria. Typical polyomavirus-like inclusions in body organs were found but these findings were not reported in the scientific literature.

1.1.3 **French Moult**

"French moult" is a condition that budgie breeders and veterinarians have recognised for many years (Perry, 1983). "French moult" was originally called "French moult" by the British because it apparently occurred in intensive breeding establishments that were set up in France late last century to raise budgerigars for the European pet market. The French called the disease "British Moult", which suggests that the disease was widespread and that nationalistic rivalry was as prevalent at the turn of the century as it is today (Smith, G., pers. comm.). In retrospect it is possible that the finger should have been pointed further south. Both France and Britain obtained their original budgerigar stocks from Australia and it is now known that viruses associated with this condition occur locally in wild psittacine birds.

Taylor (1982) reported French moult to be nonfatal but extremely disfiguring and widespread. The disease usually appeared towards the end of a breeding season, with the youngest chicks in the nest most likely to suffer problems. Primary flight and tail feathers and occasionally secondaries would be lost in a symmetrical fashion. In mildly affected birds only a few feathers would be lost and these feathers could regrow in subsequent moults. In badly affected chicks the feathers were permanently damaged. No evidence of mites or nutritional deficiencies or hereditary factors was found to be of primary importance in the development of the disease. It was noted that the disease appeared to be egg transmitted and that eggs from birds with a long history of French moult gave rise to diseased youngsters when fostered by pairs whose own youngsters, reared in the same nest, developed into perfectly healthy birds. It was also noted that birds with French moult had severely hyperplastic bone marrow with a normocytic, normochromic anaemia and fragile erythrocytes. There was also an inconclusive suggestion that high dietary levels of vitamin A could predispose birds to developing French moult.

Taylor (1982) suggested that the capillaries of the pulp of growing flight feathers are extremely sensitive to a range of adverse conditions affecting the rate of blood flow or the fragility of the capillary walls which ultimately result in vascular stasis, blood clotting and capillary rupture. Histological changes observed by Taylor (1982) suggested that French moult could be an immune-complex disease, possibly viral induced. He felt that the disease was not caused by a single agent but was likely to be multifactorial in origin.

While French moult was linked with BFD in reports from the early 1980's,

Davis (1983a, 1983b) subsequently noted that North American budgerigars serologically negative for BFD had French moult. Gerlach (1984) suggested a possible connection between budgerigarpox and French moult in Europe. Harrison (1984) suggested that environmental, hereditary, parasitic and nutritional factors could play a role in the occurrence of French moult.

Pass and Perry (1984) identified viral particles in budgerigars with French moult identical to those seen in other psittacine birds with Psittacine Beak and Feather Disease (PBFD). Subsequently Wylie and Pass (1987) demonstrated that French moult and PBFD were the same disease and due to a virus which is now been tentatively classed as a circodnavirus.

Polyomavirus-like inclusion bodies were found (Harrigan, K., pers. comm.) in fledgling budgerigar chicks presented at the author's practice and showing poor development of flight and tail feathers (typical French moult). No histological changes suggestive of PBFD were seen in these birds. The aviary in which this problem was identified did not have concurrent problems with neonatal deaths or underdeveloped chicks. Polyomavirus-like inclusion bodies in other local budgies showing poor development of flight and tail feathers were also identified (Harrigan, K, pers. comm.). Polyomavirus-like inclusions were frequently observed in budgerigars in New South Wales with French moult-like signs (Perry, R., pers. comm.).

It has been suggested that the name "French moult" be discontinued because the condition can be caused by multiple aetiologies. While this is true, "French moult" is a useful term to describe a characteristic clinical presentation. If a diagnosis has been established it would be preferable to use precise terminology.

1.1.4 **Polyomavirus in British budgerigars imported into Australia**

Polyomavirus-like inclusions were found in an adult male budgie presented at the author's practice that was amongst the first shipment of budgies imported from Great Britain into Australia through the newly established quarantine station at Spotswood, Victoria. This bird had never been well since it had arrived in Australia and was presented at the author's practice with lethargy, poor appetite, diarrhoea and intermittent vomiting. It had been treated for both a yeast and Gram-negative bacterial infection with antibiotic and antifungal treatment but showed only partial response and died some months later. Other birds in the aviary with which this budgie was housed showed no signs of disease. Megabacteriosis was found in the proventriculus, interventriculus and gizzard as well as polyomavirus-like inclusions in the kidneys. No ultrastructural studies were carried out.

While the histopathological appearance in this case was suggestive of polyomavirus, karyomegalic inclusions may occur with other conditions where there is nuclear death. If this condition was polyomavirus, whether the bird

acquired the virus after it arrived in Australia or whether he may have contracted it prior to arrival is not known. Nor was it known whether the inclusions may have been an incidental finding or whether polyomavirus may have compromised the bird's immune system, resulting in an increased susceptibility to other diseases. Perhaps other conditions may have made this bird more susceptible to viral infection .

A similar but more widespread pattern of disease was seen in a subsequent shipment of English budgerigars. A number of birds in this 1991 shipment showed varying degrees of lethargy, poor appetite, vomiting and diarrhoea. Sick birds from the shipment were presented at my practice and other veterinary practices in Victoria and elsewhere in Australia. A variety of organisms was isolated from crop samples and faecal or cloacal swabs including *Escherichia coli* and other Gram-negative bacteria; megabacteria and *Candida albicans*. Birds were treated in accordance with antibiotic sensitivity tests and in most cases showed gradual response. One budgie from my practice died in spite of treatment. On necropsy this bird showed few gross changes but on histological examination there was extensive megabacteriosis of the proventriculitis and gizzard as well as polyomavirus-like inclusions in the kidneys. These changes were similar to those seen in the bird dying from the previous shipment and reminiscent of combined infections in finches described by Johnson and Riddell (1986) and Marshall (1989). Other budgie owners anecdotally reported outbreaks of "red chicks" and French moult following the introduction of British stock to their aviaries whereas previously they had not experienced these problems.

1.2 Polyomavirus in other psittacine species

1.2.1 In psittacine fledglings (North America)

Reports of disease caused by polyoma-like viruses in psittacine species apart from budgerigars began to appear in the literature in 1983 (Graham, 1983; Clubb and Davis, 1984; Jacobsen *et al*., 1984). Jacobson *et al*. (1984) described high mortalities in fledgling macaws (*Ara spp.*) and conures (*Pyrrhura spp*.) being raised in a psittacine nursery in which affected chicks showed lethargy, loose droppings, crop stasis and dyspnoea. At necropsy affected chicks showed moderate subcutaneous haemorrhage over the crop and across the dorsum.

Histological changes were similar to those described in affected budgerigars. Large pale to lightly basophilic inclusion bodies were seen in livers and spleens. Feather abnormalities were not a feature of the disease grossly but ballooning degeneration of feather sheath epithelium was apparent microscopically in some birds.

Ultrastructural studies demonstrated polyomavirus-like particles with a mean

diameter of 42 nm. Serological examination of in-contact birds using a FAVN test showed positive titres whereas titres on birds in aviaries where there had been no contact with the sick birds remained negative.

Graham and Calnek (1987) reported polyomavirus-like infections in 44 psittacine birds of 18 species necropsied between 1982 and 1985. Conures, macaws, Amazon parrots (*Amazona spp.*), cockatoos (*Cacatua spp*.) cockatiels (*Nymphicus hollandicus*), lories (*Chalcopsitta spp*.), eclectus parrots (*Eclectus roratus*) and an African grey parrot (*Psittacus erithacus*) and a white-bellied caique (*Pionites leucogaster*) were among the species reported. Age range of the birds was between 14 days and 16 weeks and they came from 22 separate sources. Clinical histories uniformly revealed that their deaths had been sudden and unexpected or had followed only a brief (12 to 48 hour) period of weakness and lassitude, slow emptying of the crop after feeding, regurgitation of crop contents and anorexia. In one case there were subcutaneous haemorrhages and in another the stool contained blood shortly before death.

On gross necropsy typical findings included muscle pallor, slight hepatomegaly and liver discolouration, petechial and ecchymotic haemorrhages of the subcutis and/or epicardial and/or other serosal surfaces and splenomegaly. Only one of the birds had gross feather abnormalities. Microscopically karyomegaly was the most consistently observed finding. Like other authors, Graham and Calnek (1987) observed membranous glomerulopathy and hepatic necrosis sparing a narrow zone immediately surrounding periportal structures. In addition they reported lymphocyte depletion and necrosis in the medulla of the bursal follicles in 60% of cases. Karyomegaly was not associated with medullary cell necrosis.

Outbreaks of polyomavirus have not yet been reported in intensive hand rearing facilities in Australia, probably because this system of husbandry is not yet commonly practised locally.

1.2.2 **In Neophemas (Canada and Australia)**

Pass *et al*. (1984) reported a polyoma-like virus infection in two splendid parakeets (scarlet-chested parrots - *Neophema splendida*) kept in an aviary in Canada. Clinical signs in affected birds were nonspecific and similar to those described by Graham and Calnek (1987) (depression, low body weight, dyspnoea and fluid dark brownish-red faeces) but one of the two birds was 8 months, a little older than birds in previous North American reports. Gross necropsy findings included low body weight, pale small spleen and congested liver. Polyomavirus-like infections have been identified in nestling aviary bred Bourke Parrots in Melbourne (Black, D. and Madill, D., pers. comm.).

1.2.3 **In Electus Parrots (United States)**

Speer (1989) reported that polyomavirus may cause deaths in electus parrots as young as 3 weeks or as old as four and a half years. In young chicks abdominal distension, subcutaneous haemorrhage, retarded growth and in some cases abnormal feather growth were seen. In older chicks signs could be more subtle and include colic, transient gastrointestinal stasis, melena and abdominal pain. Occult haematuria was commonly seen.

1.2.4 **In African & Asiatic Parrots**

In South Africa Abrey (1984) reported deaths in fledgling (3 week-old) mutation Indian ringnecks (*Psittacula krameri*) in which typical polyomavirus inclusion bodies were found. Gill (1989) noted that he had histological diagnoses of polyomavirus-like infections in African lovebirds (*Agapornis spp*.) and Indian ringnecks (*Psittacula spp*.) in Sydney, NSW. He also described a disease syndrome in Asiatic parrots that caused deaths in nestlings within 24-48 hours of the onset of clinical signs, mortalities reached as high as 100%. Polyomavirus-like inclusions were subsequently demonstrated in affected nestlings (Gill, J., pers. comm.).

In Melbourne bone marrow necrosis and polyomavirus-like inclusions were found in Indian ringneck parrots dying subsequent to intraabdominal haemorrhage (Black, D. and Madill, D., pers. comm.).

In Western Australia, Pass (1985) reported a polyomavirus-like disease in lovebirds that occurred sporadically or in small outbreaks in aviaries and affected fully fledged birds of less than one year of age. Death of nestlings and feather abnormalities were not seen in association with the disease and lesions were limited to the kidney and liver. This condition was also seen in peachfaced lovebirds (*Agapornis roseicollis*) in Florida and serums from affected birds cross-reacted with BFD virus by indirect immunofluorescence (Gaskin, 1987).

1.2.5 **In wild psittacine birds in Australia**

In Western Australia Rowley (1990) reported on a disease syndrome in which wild nestling galahs (*Eolophus roseicapilla*) suffered weight loss, poor development, foul smelling diarrhoea, and near continuous emission of a whining call. These nestlings had full crops and continued to be fed by their parents which remained healthy and continued to breed in subsequent years. Although many nestlings died, a few recovered and fledged normally albeit later than usual. The disease lowered survival rate to less than 50% of nestlings hatched compared to 60-90% in areas where the disease was not occurring.

Subsequent work by Whitton *et al*. (1991) identified polyomavirus and enterovirus particles by electron microscopy in some affected nestlings. Villous atrophy of the duodenum and occasionally jejunum were seen in some birds necropsied. While the clinical problems of neonatal and fledgling deaths are typical of polyomavirus infection, the histological changes described were not typical of polyomavirus infections described by other authors. The examination of many birds showing typical signs of this problem did not demonstrate polyomavirus and many of the affected birds recovered provided that they were force fed (Pass, D., pers. comm.). The significance of the viruses that have been found in these birds is not known.

Enterovirus infection was described in juvenile galahs dying in Western Australia, all of which were suffering from concurrent PBFD virus infection (Wylie and Pass, 1989). A 30nm diameter virus was identified in paracrystalline arrays by electron microscopy in the faeces and in enterocytes. The virus was not isolated and transmission experiments were unsuccessful. Histological changes included villous atrophy, villous fusion, enterocyte hyperplasia and in some cases, chronic inflammation. These changes were similar to the histological picture described by Whitton *et al*. (1991) in birds from which polyomavirus and enterovirus-like particles were identified. McOrist *et al*. (1991) identified enterovirus (but not polyomavirus) in recently captured cockatoos in Victoria that were suffering from profuse diarrhoea. Histological changes were similar to those described by Wylie and Pass (1989).

Polyomavirus-like inclusions have been seen in young wild-caught sulphur crested cockatoos (*Cacatua galerita*) presented at the author's practice that have died within weeks or months of purchase. Various diseases, including PBFD, chlamydiosis, Gram-negative bacterial infections, yeast infections and megabacteriosis may also be seen in newly purchased juvenile, wild caught cockatoos and galahs. These birds are subjected to significant stress during capture and transportation and are subsequently exposed to other birds on dealers' premises that may be harbouring pathogenic organisms. Polyomavirus may be one of many contributing factors in disease outbreaks. In clinical practice, viral infections are often suspected as contributing to illnesses because affected birds often show low white blood cell counts.

1.3 Polyomavirus and other clinical signs

1.3.1 Associated with renal disease

In a retrospective study of cases submitted to a diagnostic laboratory, Phalen *et al*. (1990) reported that 34 out of 44 cases of polyomavirus infection had renal lesions. Excluding budgies, 24 out of 30 cases (80%) had renal lesions, of which, karyomegalic lesions were seen in 19 birds, primarily in glomeruli. A membranous glomerulopathy was present in 7 of the 24 (29%).

1.3.2 **Associated with neurological symptoms**

Schmidt *et al*. (1987) reported karyomegaly and polyomavirus-like inclusions in the granular cell layer of the cerebellum of a Moluccan cockatoo (*Cacatua moluccensis*) that was euthanased after showing extreme weakness, torticollis and lack of response to symptomatic treatment. The clinical picture in this bird was confused because the bird also had concurrent trematodiasis. Electron microscopy showed polyoma-like 33-38nm diameter viral particles as well as tubular structures similar to those described by Bernier *et al*. (1981). Gerlach (1986) cited posterior paresis in a double yellow headed Amazon parrot with polyomavirus, as reported by Clubb and Davis (1984).

1.3.3 **Associated with haemorrhagic disease**

Wainright *et al*. (1987) reported histological identification of polyoma-like virus in young Amazon parrots dying from a haemorrhagic disease syndrome in a quarantine station. Fifty four percent of the imported birds died. Poxvirus and reovirus were also identified in these birds. Polyomavirus-like inclusions were seen in the squamous epithelium of the crop, in the basal epithelial layer in the oesophagus and in the liver of some affected birds. Polyoma- or adeno-like viral inclusions, along with reovirus were also found in two fledgling lories dying at about 7 weeks of age. Necropsy signs included a haemorrhagic and oedematous heart surrounded by fluid, fluid-filled lungs and a swollen and mottled liver and spleen. In a third bird intranuclear inclusions were found in the bone marrow stromal cells and in the glomeruli of the kidney, both suggesting a polyomaviral origin.

1.3.4 **Associated with feather loss in adult psittacine birds**

Polyomavirus infection has been associated with feather loss in an adult rainbow lorikeet (*Trichoglossus haematodus*) (Pass, 1989).

1.3.5 **Possible association with tumours**

In mammals polyomaviruses are commonly associated with tumours. There have been no reports of tumours caused by polyomaviruses in birds but avian polyomavirus is known to transform cells in vitro (Thomas, 1988). Pass (1984) queried whether there could be any correlation between the occurrence of polyomavirus in budgerigars and the high incidence of tumours in this species. Investigations are currently being undertaken in North America to determine whether the genome of the avian polyomavirus integrates with host cell DNA, as well as to determine the site of viral latency in the carrier state. Studies are also being performed to determine whether there is any correlation between polyomavirus infections and an increased incidence of tumours (Ritchie *et al*., 1991).

1.4 Polyomaviruses in non-psittacine species

1.4.1 In galliform birds

Deshmukh *et al*. (1969) recovered a polyomavirus from the intestinal tract of a turkey with Bluecomb but the virus was not implicated as a disease causing agent.

1.4.2 In passerine birds

Polyomavirus-like intranuclear inclusion bodies have been described in finches in Canada (Johnson and Riddell, 1986), Australia (Forshaw *et al*., 1988; Marshall, 1989), Italy (Sironi and Rampin, 1987) and the United States (Woods, 1989). Polyomavirus has not yet been cultured from passerine species.

In Western Australia Forshaw *et al*. (1988) reported deaths in fledgling and immature Gouldian finches (*Erythrura gouldiae*) from three separate properties. No feathering defects were noted and the most consistent gross lesion was a swollen, pale liver which in many cases was mottled by recent haemorrhages. Microscopically there were often random areas of focal hepatic necrosis, occasionally general lymphoid depletion in the spleen, epithelial necrosis in the gizzard and mononuclear cell infiltration in the kidneys and intestines. Cells containing large, lightly basophilic or finely stippled nuclei with marginated chromatin were seen in liver, spleen, kidney, gizzard and intestine. On electron microscopy discrete, round, polyomavirus-like electron dense particles were seen. Polyomavirus-like disease has not yet been reported in wild Gouldian finches but this species has suffered significant population declines and is endangered in the wild (Tidermann, 1987).

Marshall (1989) reported polyomavirus-like infection in fostered colour mutation Gouldian finches in New South Wales. Clinically the disease was characterised by acute mortality in two to three day old babies, poor growth and beak deformity in nestlings, nonspecific illness and mortality in fledglings and young adults. In one acute outbreak 36 out of 77 nestlings died within 3 days of hatching. Of the surviving nestlings, most were small, had dirty feathers and were slow to fledge. Many of the birds had an abnormal lower mandible that was long and tubular. There was concurrent infection with *Candida albicans* in some birds. In contrast to the acute death reported in gouldians by Forshaw *et al*. (1988), the deaths in fledglings occurred over several days and were usually associated with a non specific illness characterised by weight loss, anaemia and/or bacterial or yeast infection. The organ pathology was less severe (e.g. only moderate individual liver cell necrosis) and only one or two organs contained inclusion bodies. The disease appeared to be a persistent infection complicated by secondary infections such as candidiasis.

Histopathology showed no changes in nestlings but in fledglings there was hepatocellular necrosis, diffuse enteritis and lymphocyte depletion and mild macrophage hyperplasia in the spleen. Diagnosis was based on the histological presence of large clear to amphophilic intranuclear inclusion bodies in one or more organs in the fledglings.

Johnston and Riddell (1986) described hepatic and bone marrow necrosis with karyomegalic inclusions in cordon bleu finches (*Uraeginthus angolensis*) and a tricolour nun (*Lonchura malacca malacca*) which died shortly after shipment to a pet shop in Canada. One of these birds also showed mycotic infiltration of the gizzard. The authors speculated that the birds may have been harbouring polyomavirus in latent form which was activated by the stress of transportation and that the viral infection of the lymphoreticular system may have compromised the immunological response in the bird showing gizzard mycosis.

In a case reported by Woods (1989), a painted finch (*Emblema picta*) hen that was housed with other finches died suddenly with no previously noted clinical signs. There were no other deaths in the aviary. On necropsy the bird was in good body condition but showed hepatomegaly and partial obstruction at the cloaca with inspissated faecal matter and urates. Histopathology showed hepatocellular necrosis, myocarditis and inflammatory changes in other body organs. Karyomegalic inclusions, similar to those reported in psittacine species, were seen in the liver, kidney, spleen, pancreas and the heart. The karyomegalic cell nuclei fluoresced using polyclonal antibody FITC-labelled conjugate specific for polyomavirus antigen. Electron microscopy revealed polyomavirus-like particles and occasional aberrant elongate forms.

1.5 Native and Exotic Animal Pathology Register reports

Reviewing unpublished data from the Native and Exotic Animal Pathology Register (NEA Path Register) it is apparent that polyomavirus-like inclusion bodies have been identified in a wide variety of avian species (Hartley, W., pers. comm.). This register is kept at Taronga Park Zoo in Sydney but contains material submitted from all over Australia. Species in which karyomegalic inclusions were identified include:

Psittacine species:

* Peachfaced and Masked Lovebirds (*Agapornis personata*). Inclusions were seen in lung, liver, kidney, small intestine, spleen. Birds came from New South Wales, Western Australia and Victoria.
* Sulphur Crested Cockatoo. Inclusions were seen in the kidney. The bird came from New South Wales.
* Galahs. Inclusions were seen in kidney and liver. Birds came from South Australia and Western Australia.

* Alexandrine Parrot (*Psittacula eupatria*). Inclusions were seen in kidney, spleen and liver. The bird came from NSW.
* Kakariki (*Cyanoramphus spp*.). Inclusions were seen in the kidney. The bird came from NSW.
* Macaw. Inclusions were seen in the kidney. The bird came from NSW.

Non-psittacine species:

* Peaceful Dove (Diamond dove - *Geopelia cuneata*). Inclusions were seen in the spleen. The bird came from Victoria.
* Brown Pigeon (Pink-breasted Cuckoo-Dove - *Macropygia amboinensis*). Inclusions were seen in the liver. The bird came from Victoria.
* Star finch (*Neochmia ruficauda*), Gouldian finch, canary (*Serinus canaria*) and unidentified finches. Inclusions were seen in kidney, liver, gizzard and spleen. Birds came from NSW, WA, Victoria and Queensland.

1.6 Epidemiology

Both horizontal and vertical transmission of polyomavirus has been demonstrated in budgerigars (Bernier *et al*., 1981; Taylor, 1982). It appears that the virus may cause persistent infections in certain individual birds that shed virus in urine and faeces, especially during times of stress or hormonal influences. Polyomaviruses are very resistant and persist in contaminated environments.

Recent work involving the use of DNA-based diagnostic tests have modified prevailing views on the epidemiology of avian polyomavirus. While some birds develop neutralising antibodies in response to polyomavirus this is not always the case and there is no concordance between virus neutralising antibody titres and viral shedding. In studies by Niagro *et al*. (1991), transient shedding of the virus was the usual pattern, with only 3 birds of 41 still shedding virus 60 days after infection.

Work by Phalen *et al*. (1991) suggested that in budgerigars reproductive changes enhanced virus replication. Breeding budgies had higher concentrat-ions of polyomavirus in various body organs when compared with non breeding budgies. It was postulated that the mechanism for this could involve a negative effect of oestrogens, progesterone or testosterone on cell mediated immunity. Resting birds between breeding cycles and limiting the number of clutches were suggested as ways of reducing the effects of BFD, although the procedure would not eliminate the virus.

Concurrent active infection with both avian polyomavirus and PBFD has been

demonstrated in budgerigars, peachfaced lovebirds, an African grey parrot and an umbrella cockatoo (Phalen *et al*., 1991). Transient shedding of polyomavirus was also documented in cockatoos with PBFD. It was suggested that PBFD through immune suppression could permit reinfection or reactivation of polyomavirus infection in affected birds.

1.7 **Diagnosis**

A number of disease conditions have been associated with avian polyomaviruses:

a. Deaths in nestling and fledgling budgerigars (Bernier *et al*., 1981).
b. Feather dystrophy in fledgling budgerigars (Bernier *et al*., 1981).
c. Deaths in fledgling psittacine birds of species other than budgerigars (Graham *et al*., 1987).
d. Renal disease, diarrhoea and haemorrhagic disorders in psittacine birds (Wainright *et al*., 1987).
e. Neurological disorders in psittacine birds (Schmidt *et al*., 1987).
f. Feather dystrophy in adult psittacine birds (Pass, 1989).
g. Sudden death in adult psittacine birds and finches (Johnson and Riddell, 1986; Pass *et al*., 1984; Ritchie *et al*., 1991b).
h. Nestling and fledgling mortality in finches (Marshall, 1989).
i. Apparent immunosuppression leading to secondary bacterial and fungal infections (Johnson and Riddell, 1986).
j. Beak abnormalities in nestling finches (Marshall, 1989).

It is not possible to diagnose polyomavirus infections on clinical signs alone. Concurrent infections with other diseases are likely. The identification of large, clear to slightly basophilic intranuclear inclusion bodies ("karyomegalic inclusions") in body organs is the standard current diagnostic method, backed up with virus specific antibody staining (Graham *et al*., 1987) and the identification of polyomavirus-like particles on electron microscopy.

It is not known whether all conditions associated with polyomavirus in birds are caused by the same virus or whether several avian polyomaviruses may be involved. So far psittacine polyomaviruses appear to be antigenically and morphologically related. DNA sequence homology in the major antigen sequence has been demonstrated from numerous isolates (Ritchie *et al*., 1991b). However, the virus as it occurred in budgies was easy to isolate while workers found it extremely difficult to isolate the virus from other species showing apparently the same or very similar lesions (Graham, 1983). Transmission studies are needed to establish whether the virus identified in finches is the same as that found in psittacine birds. The significance of polyomavirus-like infections in species other than psittacine birds and finches needs to be investigated.

Virus isolation and experimental transmission have been performed in a few species (Bernier *et al*., 1981; Lynch *et al*., 1984) but may be difficult and time consuming to perform. While there are no published reports of polyomavirus being cultured in Australia, it has been cultured on two occasions in Western Australia and transmitted to lovebirds (Pass, D., pers. comm.).

None of the above diagnostic techniques are suitable for mass screening of live birds. Detection of circulating antibodies to the virus in blood of adult birds has been performed by means of immunodiffusion (Lynch *et al*., 1982), fluorescent-antibody virus-neutralisation (Wainright *et al*., 1987b), indirect immunofluorescence (Gaskin, 1988) and plaque reduction assay. Overseas viral neutralising titres have been used commercially to indicate birds which could be shedding polyomavirus but recent work using a DNA-based diagnostic test has demonstrated that this test is an unreliable indicator of virus shedding (Gaskin, 1990; Niagro *et al*., 1990; Ritchie *et al*., 1991a).

While not yet commercially available, a polymerase chain reaction coupled with a DNA probe for polyomavirus shows promise of being able to detect virus in small samples of whole blood or faeces or in tissues (Niagro *et al*., 1990; Ritchie *et al*., 1991a). The genome of BFD has been characterised (Rott *et al*., 1988). Initial work showed difficulties in detecting the virus in serum samples or in tissues fixed in formalin. Uric acid in faeces may also interfere with the PCR reaction. Work is currently continuing on improving techniques. Preliminary results from the test can be obtained with 24 hours of sample submission (Ritchie, BW, pers. comm.).

1.8 **Differential diagnosis**

Psittacine beak and feather disease.

Feather abnormalities caused by polyomavirus need to be distinguished from those caused by PBFD. PBFD does not typically cause high nestling mortalities. The clinical appearance of cockatoos with advanced PBFD is typical and, with experience, this disease can be strongly suspected on clinical examination of an affected bird. Clinical appearance is often less distinctive in psittacine species other than cockatoos (Pass and Perry, 1984; Ritchie et al., 1990a). Histologically birds with PBFD typically have necrosis of feather epidermal cells and macrophages containing purple intracytoplasmic inclusion bodies in affected epidermis and feather pulp. In some birds positive for PBFD large intranuclear inclusion bodies resembling those seen with polyomavirus infections have also been seen due probably to dual infections. A confirmatory diagnosis requires the use of viral specific antibodies or DNA probes to differentiate between PBFD and BFD virus Ritchie *et al*., 1990a). A haemagglutination inhibition test has been developed for PBFD. On electron microscopy PBFD virus

particles are nonenveloped, icosahedral and have a mean diameter of 14-16nm, much smaller than polyomavirus.

Pacheco's Disease or other avian herpes viruses.

Feather dystrophy is not typically associated with Pacheco's Disease, nor do mortalities selectively affect nestlings (Gerlach, 1986). Pacheco's Disease has not yet been reported in Australia although herpesvirus-like inclusions have occasionally been identified histologically in psittacine birds.

Budgerigar herpesvirus.

This has been reported in Europe associated with feather dystrophy. This virus is predominantly found in feathers and is rare in other organs, blood or faeces, it is egg transmissible and may cause reduced hatchability. Pigeon herpes has been isolated from budgerigars and has experimentally produced disease in this species (Gerlach, 1984). Herpesvirus has been associated with papillomatous foot lesions on the legs of cockatoos. (Lowenstine *et al*., 1983).

Differentiation of diseases caused by herpesvirus from those caused by polyomaviruses relies on history, histological appearance (herpesviruses are typically associated with Cowdry type A, eosinophilic intranuclear inclusion bodies) electronmicroscopic identification of characteristic viral particles (herpesviruses are enveloped and vary in diameter from 120 to more than 200nm) and virus isolation (Creager *et al*., 1990).

Adenovirus(es) have caused hepatitis, pancreatitis and unapparent infections in budgerigars and lovebirds. Typically adenoviruses produce basophilic intranuclear inclusion bodies and will grow and produce cytopathic effects in continuous cell lines such as HeLa cells. On electron microscopy adenoviruses are icosahedral, 70 to 90 nm and not enveloped (Jacobsen *et al*., 1989; Creager *et al*., 1990). Adenovirus-like infections have been identified in psittacine birds, particularly in lovebirds, in Australia.

Reovirus has been isolated from psittacine birds suffering from hepatitis, enteritis and multifactorial disease syndromes. The virus may be associated with lesions such as hepatomegaly and splenomegaly (Clubb *et al*., 1985). It does not produce inclusion bodies. Psittacine reoviruses have not yet been reported in Australia.

Cytomegalovirus causes depression, anorexia, dyspnoea, conjunctivitis and a mortality rate of 70% has been associated with cytomegalovirus-like particles in Australian finches in Europe. On histopathological examination the birds showed numerous intensely basophilic inclusions in karyomegalic epithelial

cells. Ultrastructural studies showed both enveloped (135 nm) and non enveloped (80nm) viral particles (Desmidt *et al*., 1991). Polyomaviruses are never enveloped and measure approximately 30 to 56 nm, smaller than cytomegalovirus. On histopathological examination polyomavirus inclusions are amphophilic or only slightly basophilic rather than intensely basophilic and inclusions may be seen in many body organs rather than localising in epithelial cells. Cytomegalovirus-like inclusions have been noted in birds submitted to the NEA Pathology Register but their role in clinical disease in avian species in Australia is not clearly defined.

Chlamydiosis is rarely associated with feather dystrophy or mortalities selectively affecting juveniles but it may be associated with adult mortalities and immunosuppression leading to secondary infections. Concurrent chlamydia/polyomavirus infections are possible.

Bacterial infections, fungal infections, poor management. Problems in these areas may be associated with polyomavirus infections or may result in similar clinical presentations such as neonatal mortalities, feather abnormalities and deaths in adult birds. History may give some indications as to the likelihood of problems in management. Gram-staining and bacterial and fungal cultures may indicate the presence of abnormal gastrointestinal tract flora. None of these organisms would be expected to cause karyomegalic inclusions, nor would viral particles be visible on ultrastructural examinations, nor would there be serological evidence of polyomavirus.

1.9 Control

There is no specific treatment currently available for avian polyomavirus infections. Supportive treatment with injectable vitamin K1 may be helpful in cases where haemorrhage is seen and can affect survival rates favourably (Speer, 1989). Treatment for concurrent bacterial or fungal diseases based on culture and sensitivity testing may be useful. Research aimed at developing an effective vaccine against polyomavirus is occurring in several centres. An experimental vaccine shows promise in the control of the disease but it is not yet commercially available (Rosskopf, 1989; Ritchie *et al*., 1991a).

1.9.1 Disinfectants for use against papovavirus.

Papovaviruses, like reovirus, are hydrophilic viruses that do not combine with lipids. Hydrophilic viruses are more difficult to kill with disinfectants than are lipophilic viruses such as herpes or influenza. Disinfectants that are effective against hydrophilic viruses include chlorinated compounds such as sodium hypochlorite (bleach) or chloramine, 70% ethyl alcohol and glutaraldehydes. Disinfectants that are not effective against hydrophilic viruses include iodines, quaternary ammonium compounds, phenols and chlorhexidine gluconates (Clipsham, 1990). However, little specific data on the susceptibility of avian polyomavirus is available.

1.9.2 **Management procedures**

Care should be exercised in the management of parrot nurseries to ensure that each set of clutch mates is physically and managerially isolated from other broods in the nursery. Each brood should have its own set of feeding implements which are washed and sterilised between feedings. The person feeding the nestlings should exercise care to avoid any form of infectious contamination between the young birds. Circumstantial evidence suggests that eclectus parrots and some conures may play a carrier role in the spread of the disease. Direct and indirect contact with breeding populations of budgerigars should be avoided by aviculturists rearing other species of parrots (Graham, 1987).

Interrupting breeding for several months has been suggested as a way to reduce problems with the disease in budgerigars (Phalen *et al*., 1991).

2 PAPILLOMAVIRUSES

Papillomavirus is the other genus of *Papovaviridae* that has been reported to cause disease in birds. Cutaneous papillomas have been reported in Amazons, African greys, cockatiels and budgerigars (Lena *et al*., 1973; Petrak and Gilmore, 1982; Jacobsen *et al*., 1983; Van der Heyden, 1988). 2The incidence appears to be low, between 0.5%-3% of tumours reported in several studies reviewed by Petrak and Gilmore (1982) were papillomas. It is important to note that papillomatosis is only a histological diagnosis. The aetiology for many papilloma-like lesions remains unknown. Very few papillomas in birds have yet been demonstrated to be viral associated (Lena *et al*., 1973; Jacobsen *et al*., 1983).

Avian papillomavirus was demonstrated in association with papillomas on the legs of European Chaffinches (Lena *et al*., 1973; Osterhaus *et al*., 1977). Three hundred and thirty birds out of approximately 25,000 birds examined were affected. The virus was purified and its physicochemical properties characterised.

Jacobsen *et al*. (1983) demonstrated papillomavirus in a single imported African grey parrot with cutaneous papillomatosis around its beak and eyelids using both immunoperoxidase screening of paraffin sections for papillomavirus group-specific antigens and by transmission electron microscopy. This individual was the only bird observed to have lesions out of more than 5,000 African Greys examined. One of this bird's warts became almost as big as the bird's head and was surgically removed seven times but continued to recur. When the growth interfered with the muscles of mastication the bird was euthanased. There had been no response to an autogenous wart vaccine (Gaskin, 1986).

2.1 Australian Experiences with Papillomavirus

To date papillomavirus has not been identified in birds in Australia. Wart-like lesions are occasionally seen on feet and legs of galahs and cockatoos in Australia. Both flat white lesions, grossly similar in appearance to lesions thought to be caused by herpesvirus (Lowenstine *et al*., 1983) as well as raised, circumscribed proliferative lesions are seen. The author has removed both types of lesions surgically in a limited number of cases and has not encountered problems with regrowth. Ultrastructural studies are currently being undertaken at the Victorian Institute of Animal Science to determine whether these lesions may be virus-associated. Topical anti-herpes virus ointments may be useful in the treatment of herpesvirus induced warts in psittacine birds.

The author has also noted papillomatous lesions on the plantar surface of both feet of wild Australian magpies (*Gymnorhina tibicen*) that have been too extensive to remove surgically. Perry has seen similar cases (Perry, R., pers. comm.). Further studies are needed to determine the cause of these lesions.

2.2 Differential Diagnosis

Papilloma-like lesions have occasionally been associated with chemical carcinogens and migrating nematodes.

Cloacal, oral and internal papillomas - "Internal papillomatous disease" appears to be common in captive psittacine birds both in Europe and North America (Van der Heyden, 1988), but no causative agents have yet been isolated or observed in association with these papillomas in parrots. Sunderberg (1984) used immunoperoxidase screening of avian papillomas for papillomavirus capsid antigens but was unable to identify virus in 25 birds with cloacal papillomas. He noted that papillomaviruses cause lesions of the squamous epithelium and since the cloacal lesions are of nonsquamous epithelium, this could account for the lack of evidence for the virus. Papillomavirus may be present as episomal or integrated DNA as is the case with equine sarcoids and some human carcinomas. DNA studies are necessary to try to determine if this is the case. Cloacal, oral and internal papillomas have not yet been reported in birds in Australia.

Poxvirus may be associated with papilloma-like lesions on the head and feet and elsewhere on the body. Eosinophilic intracytoplasmic inclusion bodies (Bollinger bodies) can usually be demonstrated microscopically with poxvirus infections. Avian poxviruses have not been identified in psittacine birds in Australia but they do occur in other birds of other orders.

Herpesvirus has been reported by Lowensteine *et al*. (1983) to be associated with papilloma like lesions on the feet and legs of cockatoos. Herpesvirus like particles were identified by electron microscopy.

3 Discussion

Understanding of the extent and nature of papovavirus infections has been hampered in Australia by the unavailability of definitive diagnostic tests for either ante-mortem or necropsy samples. The use of PCR and DNA probes is being developed for diagnostic testing overseas but the test is complex and will require specialised laboratory facilities. The identification of characteristic karyomegalic inclusions has been the basis for tentative diagnosis but without follow up use of electron microscopy, immunoperoxidase staining or DNA probes it is not possible to make a definitive diagnosis. Economic constraints of avian practice in Australia are such that many owners will decline even the cost of histopathology. It is likely that many cases of polyomavirus associated disease go undetected.

Avian papovaviruses are not known to cause significant disease in commercial poultry or humans. While losses for budgerigar breeders have been a problem over many years, the economic impact in this species was initially low because the cost per individual budgie was low. This situation has changed as the value of aviary birds has increased, with the advent of legalised avian importation into Australia and with the increase in intensive rearing practices. While other diseases may result in high mortalities, polyomavirus losses are often more insidious and may continue over a long period because of the carrier status of some birds. Breeding stock that owners have worked years to develop may not necessarily die but may show increased susceptibility to disease and their offspring may have increased mortality. Birds sold to other establishments may carry virus and be the source of disease problems. Some clients have chosen to stop breeding birds once they have experienced major problems with polyomavirus rather than face ongoing problems with disease or depopulation and restocking with no guarantee that new stock will be virus free.

The identification of polyoma-like viral particles in the droppings of wild galahs in Western Australia is the first report of this virus in wild birds from anywhere in the world. While clinical evidence suggests that the virus may occur in wild birds in other parts of Australia this has not yet been proven.

Papillomavirus was identified from wild chaffinches (???) in Britain and the imported African grey with papillomavirus in the one North American report was presumably wild and of African origin. Papillomavirus has not yet been identified in Australia but clinical cases suggestive of papillomavirus occur and the condition may be undetected because of poor diagnostic follow up. With the increase in captive breeding and reintroduction programs care needs to be taken that these viruses are not inadvertently introduced to previously unexposed wild bird populations.

It is possible that polyomavirus could have been introduced into Australia by importation of exotic birds decades ago but it seems more likely that the disease has occurred here naturally since before European settlement. There have been no published reports of polyomavirus in *Columbiformes* but there were pathology reports of typical karyomegalic inclusions from a peaceful dove and a brown pigeon in the NEA Pathology Register as well as records of infections from many species of psittacine birds

and finches. Further studies are needed to determine whether polyomavirus-like infections in non-psittacine birds are caused by the same virus that has been identified in psittacine birds.

Polyomavirus is not exotic to Australia but circumstantial evidence suggests that birds going through the quarantine system may harbour the virus and may be responsible for the introduction of the virus into aviaries that had previously been free of the disease. Owners that have imported budgerigars into Australia that have subsequently had polyomavirus-like infections diagnosed in their aviaries have expressed anger that the long awaited birds for which they had paid dearly and planned carefully had proven to be a disappointment and that the birds could place their own domestically bred stock at risk. They have also expressed disappointment that the quarantine system was ineffective in preventing disease introduction.

Quarantine is ineffective when dealing with viral carrier states. Crowding birds from different sources in confined, stressful surroundings could predispose to viral shedding from affected carrier birds and these birds could spread the infection to other previously unexposed individuals. The quarantine station itself could contribute to the spread of this disease rather than preventing its occurrence. Polyomavirus might well play a role in suppressing immunity of birds in the quarantine station and allow other disease causing agents to multiply.

Egg importation is not an appropriate approach to eliminating this virus as polyomavirus is egg transmitted. Importation of avian semen could be a possible alternative but freezing semen and artificial insemination are not yet widely used in birds and it is likely that polyomavirus could be semen-transmitted.

Recent and Future Developments

Polyomaviral DNA probes have just become commercially available in the United States and are being promoted to detect polyomavirus in cloacal swabs of birds that are actively shedding the virus, for screening breeding flocks and in detecting polyomavirus in necropsy material. DNA probe technology is already advanced in Australia and local application of this technology to diagnosing polyomavirus infections should be possible.

Phalen *et al*. (1992) collected evidence to demonstrate that polyomavirus infection is not synonymous with development of disease. They suggested that the severe fatal manifestations of polyomavirus disease may prove to be immune-mediated, that antihistamine treatment may be useful in treating birds suspected of having polyomavirus disease and that the complex nature of the disease may make development of an effective vaccine difficult. In spite of these difficultuies, development of an effective vaccine is currently a top priority and work is progressing in several centres to achieve this.

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