

Polyomavirus encephalopathy in a Ducorps cockatoo (*Cacatua ducorpsii*) with psittacine beak and feather disease

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Abstract. Necropsy tissues were examined from an adult wild-caught Ducorps cockatoo (*Cacatua ducorpsii*) with progressive neurologic signs. Of the tissue specimens selected for histologic evaluation, only the brain contained rare amphophilic, glassy intranuclear inclusions within astrocytes and some neurons. Astrocyte and neuronal degeneration and necrosis also were observed. Scattered astrocytes, with and without discernable inclusions, contained avian polyomavirus (APV) nucleic acid, as determined by DNA in situ hybridization. In addition, endothelial cells and intravascular leukocytes contained psittacine beak and feather disease viral nucleic acid, as determined by DNA in situ hybridization, indicating dual viral infection. Electron microscopic examination of formalin-fixed brain tissue revealed typical intranuclear APV particles in some astrocytes. Encephalopathy ultimately was attributed to APV infection.

Avian polyomavirus (APV), also known as budgerigar fledgling disease virus, is a member of the family Papovaviridae. APV is a 40-50-nm-diameter icosahedral, nonenveloped virion containing a 4,981-base-pair circular, double-stranded DNA genome.^{5,19} Polyomavirus infection in psittaciform birds may be subclinical or may present as a fatal, acute, multisystemic disease. Although observed more frequently in budgerigars, APV infection may result in death of both juvenile and adult nonbudgerigar psittacine species.¹⁶

Histologically, systemic infection with APV in budgerigars and parrots usually is associated with large amphophilic nuclear inclusions in a number of tissues, especially spleen, liver, and kidney. Marked splenic and hepatic necrosis also may be observed. Viral inclusions also have been observed microscopically in brain tissue;^{1-3,6} however, reports of virus-induced neurologic dysfunction are rare. Tremors of the head, neck, and limbs, incoordination, and ataxia have been described in nestling budgerigars with APV infection. Approximately 10% of susceptible neonates exhibited neurologic signs.¹³ In addition, a Moluccan cockatoo (*Cacatua moluccensis*) with APV infection exhibited inability to perch properly and had slight torticollis that progressed to a semicomatose state.¹⁷ Neurologic deterioration was observed over a 2-day period following a 1-week history of illness.

The purpose of this report is to describe concurrent APV and psittacine beak and feather disease virus (PBFDV) infections in a Ducorps cockatoo (*Cacatua ducorpsii*) with a primary complaint of progressive central nervous system (CNS) dysfunction. The disease in this bird is compared with combined polyomavirus encephalitis and human immunodeficiency virus (HIV) infection.

Materials and methods

Case history. An adult Ducorps cockatoo was obtained from a group of approximately 50 wild-caught birds of similar species. These birds were held in quarantine in Hawaii for 90 days. During this period, a number of birds were observed to have or to develop clinical signs of psittacine beak and feather disease (Pbfd), most notably feather dystrophy and loss. Following release from initial quarantine, the bird was purchased and transported to California where it was held in quarantine for 3 additional weeks. Feather abnormalities suggestive of Pbfd were not observed; however, progressive neurologic signs developed, including body tremors and unsteadiness on the perch. Clinical signs of CNS disease were present for less than 1 wk.

Neither biochemical nor hematologic abnormalities were detected. The cockatoo's neurologic disease was unresponsive to empiric administration of cephalosporin, tending to exclude bacterial encephalitis. The bird ultimately was found dead in its enclosure. The cagemate was negative for PBFDV and APV infections on molecular testing and exhibited no overt clinical signs of either disease.

The dead cockatoo was necropsied by the referring veterinarian. Sections of brain, spleen, liver, kidney, heart, and lung were submitted in 10% neutral-buffered formalin for histologic examination.

Further epidemiologic investigation revealed that the cockatoo of this report had been held in quarantine with

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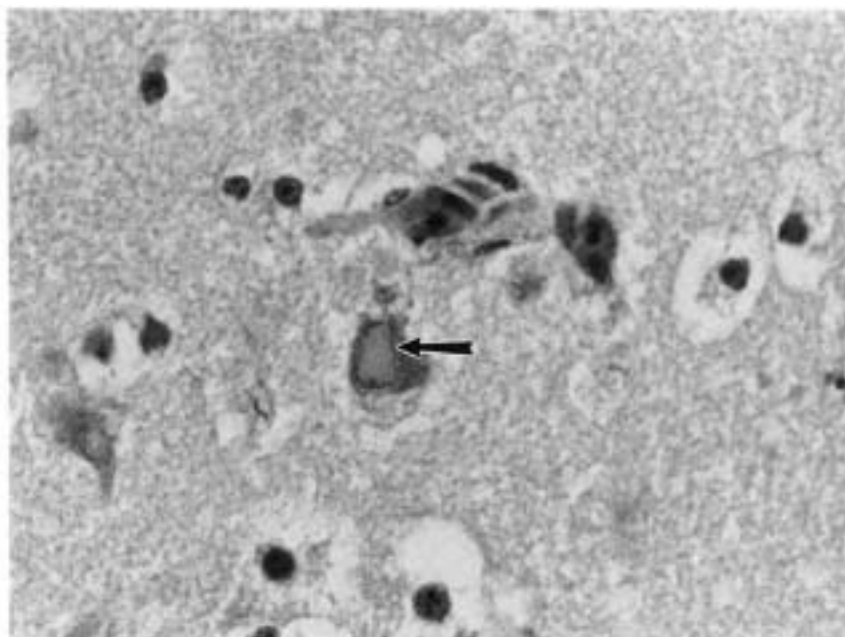


Figure 1. Cerebrum; Ducorps cockatoo. Intranuclear avian polyomavirus inclusion (arrow) in a neuron. Scattered degenerating astrocytes also are present. HE.

several other adult birds of the same species, 1 of which had dystrophic feathers but did not develop neurologic signs. Subsequent feather biopsies of that bird documented progressive PBFDV infection and the bird was euthanized. Furthermore, DNA in situ hybridization of necropsy tissues collected from that same bird documented concurrent PBFDV and APV infections.⁸

Tissues and DNA in situ hybridization. Formalin-fixed necropsy tissues were processed routinely, embedded in paraffin, sectioned at 3 μ m, stained with hematoxylin and eosin (HE), and examined microscopically. Additional sections of brain were subjected to luxol fast blue staining to evaluate possible demyelination.

Replicate tissue sections of brain were placed on charged and precleaned glass microscope slides^a and processed for DNA in situ hybridization to demonstrate APV and PBFDV nucleic acids following published techniques.⁸ A work station^b was used to simplify manual procedures, control reaction temperatures, and minimize reagent consumption. Tissue sections were dewaxed and rehydrated by passage through graded alcohols to automation buffer^c and were then digested with 0.3% pepsin. Prehybridization was performed with 100% formamide treatment. Tissue sections were then hybridized to digoxigenin-labeled virus-specific (PBFDV or APV) DNA oligonucleotide probes for 5 min at 110 C and then for 30 min at 37 C. Following stringency washes in standard saline citrate, anti-digoxigenin antibody conjugated with alkaline phosphatase was applied to the tissue sections. The chromagen was nitroblue tetrazolium dye. Tissue sections were counterstained with fast green, dehydrated, coverslipped, and examined microscopically.

Both positive and negative control tissue sections were used to validate the DNA in situ hybridization procedures.

Deposition of dark blue-black pigment (reduced formazan) indicated the presence of viral DNA.

Electron microscopy. Formalin-fixed portions of brain were diced into 1-mm cubes and placed in Trump's fixative. Tissue specimens were postfixed in 1% phosphate-buffered osmium tetroxide (pH 7.2), dehydrated in graded alcohols, and embedded in Spurr low-viscosity resin. Thin sections were placed on formvar-coated high-transmission nickel grids, stained with methanolic uranyl acetate and Reynolds's lead citrate, and examined with a transmission electron microscope.

Results

Examination of HE-stained tissue sections revealed various microscopic changes within the gray matter. Scattered astrocytes and fewer neurons appeared degenerative to necrotic. These cells were shrunken and had eosinophilic cytoplasm; karyorrhexis was observed occasionally. Some astrocytes and neurons also contained glassy, amphophilic intranuclear inclusions typical of APV (Fig. 1). Nuclei containing inclusions were of normal size or slightly enlarged. Other astrocytes had swollen nuclei with chromatin clearing, but classical nuclear inclusions were not observed. Inflammatory cell infiltrates were not present. Cerebral blood vessels were congested. Accentuated spaces around vessels and microglial cells were interpreted as processing-induced tissue shrinkage artifacts. Luxol fast blue staining failed to disclose demyelination.

DNA in situ hybridization demonstrated APV nucleic acid within some astrocyte nuclei, including cells

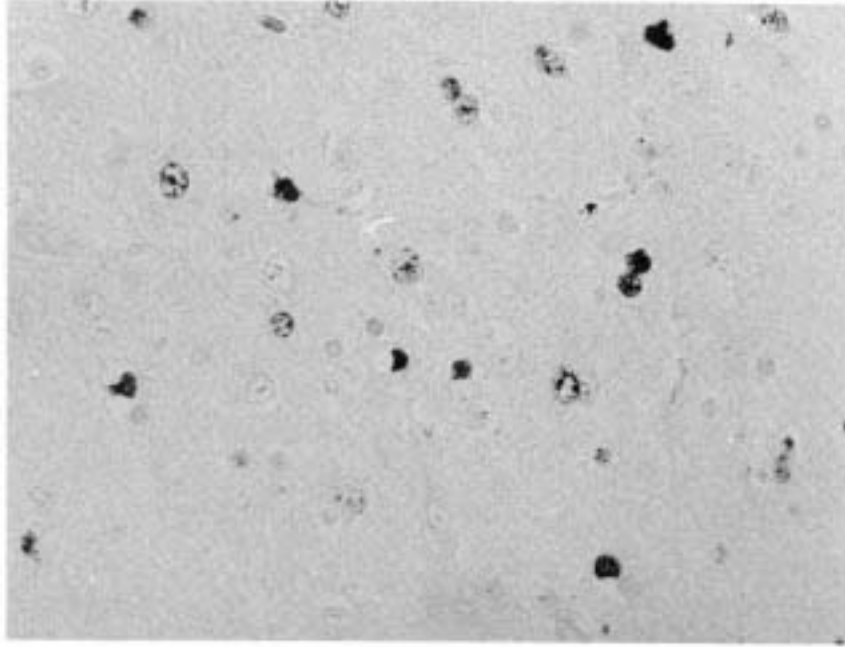


Figure 2. Cerebrum; Ducorps cockatoo. DNA in situ hybridization demonstrating avian polyomaviral nucleic acid within astrocyte nuclei. Anti-digoxigenin-alkaline phosphatase-NBT system with fast green counterstain.

with and without nuclear inclusions in corresponding HE-stained tissue sections (Fig. 2). In addition, DNA in situ hybridization detected PBFDV nucleic acid within some circulating leukocytes and cerebral endothelial cells, documenting concurrent viral infection.

Transmission electron microscopy revealed 45-50-nm-diameter virions within the nuclei of some astrocytes (Fig. 3). Although preservation of the tissue was not ideal because of initial fixation in 10% formalin solution, virions were identified readily. Both the mor-

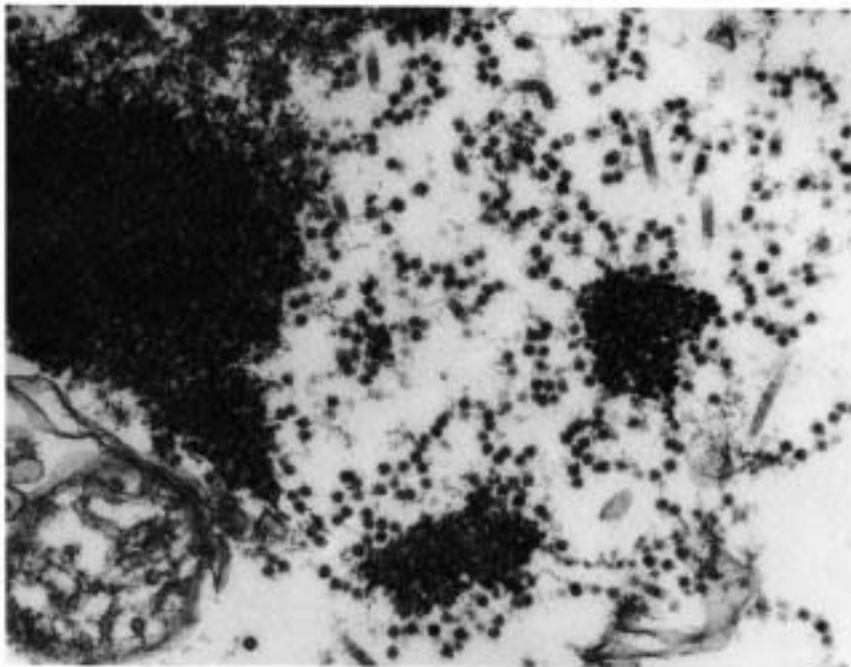


Figure 3. Cerebrum; Ducorps cockatoo. Avian polyomavirus particles within the nucleus of an astrocyte. Uranyl acetate and lead citrate.

phology and size of the virus particles were consistent with APV.

Discussion

Viral encephalopathy is observed rarely in companion and free-ranging birds. Neurologic disease has been associated with polyomavirus infection of nestling budgerigars (*Melopsittacus undulatus*)¹³ and one adult Moluccan cockatoo.¹⁷ In addition, CNS disease or encephalitis has been described following experimentally induced or natural outbreaks of paramyxovirus infection in half-moon conures (*Aratinga canicularis eburnirostrum*), yellow-headed Amazon parrots (*Amazona ochrocephala oratrix*),⁴ parakeets (*Neophema* sp.),¹⁸ and a Moluccan cockatoo.¹¹ In contrast, PBFVDV infection has not been associated with neurologic signs or encephalitis.

APV is associated with formation of glassy, amphiphilic nuclear inclusions in the brain.^{1-5,13,17} These inclusions have been observed most frequently in the granular, molecular, or Purkinje layers of the cerebellum or in the glial cells of the cerebellar and optic lobe white matter.^{1,13,17} Although cerebellum was not present in the tissue specimens submitted from this cockatoo, inclusions were confined to astrocytes and scattered neurons in the cerebral cortex. In contrast to APV, paramyxovirus may cause formation of single to multiple, round to oval, eosinophilic to amphiphilic, cytoplasmic inclusions of various sizes within neuronal cell bodies. Nuclear inclusions also may be observed in neurons, glial cells, and syncytial cells.¹¹ Paramyxovirus infection often is associated with gliosis, neuronophagia, and mononuclear cell perivascular cuffing, which have not been reported in APV infection.¹⁸ Viral inclusions may be observed in brain tissue during systemic APV infection; however, neurologic manifestations of disease generally are not reported.^{1-3,6} These observations suggest that APV infection of the CNS occurs frequently, but viral encephalopathy is rare.

High concentrations of APV DNA recently have been reported in the brains of clinically healthy budgerigars. This study used the polymerase chain reaction (PCR) to amplify and detect APV DNA in brain tissue of 18 of 20 birds examined.¹⁴ These results should be interpreted with caution because approximately 43% of those same birds also had detectable APV DNA in the serum. Therefore, the presence of PCR-amplified APV DNA in the tissue or organ homogenates (including brain) is expected because each specimen would be contaminated with blood. Consequently, confirmation that APV DNA really exists in extravascular brain tissue of clinically healthy birds will require tissue-level detection by DNA in situ hybridization and/or DNA in situ PCR techniques.

In human beings, JC virus (a polyomavirus) exhibits distinct neurotropism, infecting both astrocytes and oligodendrocytes. Infection of oligodendrocytes results in a progressive, multifocal demyelination, which is reflected clinically as progressive CNS disease. Although JC virus may cause CNS disease in immunocompetent people, it has a greater propensity to cause encephalopathy in patients with an underlying disease that impairs cellular immunity.¹² This situation occurs in patients with HIV infection, wherein opportunistic infection with JC virus is observed in approximately 3.6% of the population.^{15,20}

The neurologic disease in the cockatoo of this report was associated with concurrent APV and PBFVDV infection, which has been documented previously in psittaciform birds.⁸ PBFVDV infection often is associated with clinical evidence of acquired immunodeficiency, leading to a variety of secondary or opportunistic infections.^{7,9,10} The APV infection in this bird was distinctly neurotropic; inclusions were not observed in the common target organs (liver, spleen, kidney). Progressive neurologic disease probably resulted from virus-induced damage to astrocytes and neurons, but the specific mechanism of cellular injury is unclear. Because the cerebellum was not submitted for examination, the origin of the body tremors could not be evaluated fully. Oligodendrocytes apparently were uninfected, which explains the lack of demyelination, such as that observed in JC virus infections of humans.

Sources and manufacturers

- a. ProbeOn Plus slides, Fisher Scientific, Pittsburgh, PA.
- b. Microprobe work station, Fisher Scientific, Pittsburgh, PA.
- c. Biomedica Corp., Foster City, CA.

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