**Sources and manufacturers**

a. Genosys Biotechnologies, The Woodlands, TX.  
b. Pharmacia, Piscataway, NJ.  
c. Life Technologies, Gaithersburg, MD.  
d. Promega, Madison, WI.  
e. Precision Scientific, Chicago, IL.

**References**

1. Davis VS, Boyle JA: 1990, Adapting the polymerase chain reaction to a double-stranded RNA genome. Anal Biochem 189: 30-34.
5. Higuchi R: 1989, Rapid, efficient DNA extraction for PCR from cells or blood. Amplifications 2:1-3.

**Budgerigar fledgling disease (papovavirus) in pet birds**

Richard S. Kingston

Budgerigar fledgling disease (BFD), a contagious disease causing high mortality in fledging budgerigars (*Melopsittacus undulatus*), was first reported in the United States and Canada and subsequently has been recognized in other countries. The causative agent was identified as a papovavirus and has been characterized as the first nonmammalian member of the polyomavirus genus. In addition, papovavirus infection of numerous other bird species has been described. This report describes BFD in a small aviary and briefly reviews papovavirus infection in pet birds.

Seven budgerigars, 20-30 days old, were presented for diagnostic evaluation. Beginning 6 months earlier, mortality in young budgerigars had markedly increased from < 10% to >90%. The aviary owner reported that young birds most often died between 10 and 14 days of age (range, 10-28 days), and those that survived were stunted and lacked normal feather development. Adult budgerigars, however, all appeared normal and were producing a normal number of fertile eggs.

The affected budgerigars were small when compared with unaffected age-matched nestlings. They lacked filoplumes on the head and neck and down feathers on the back and breast and had underdeveloped primary and secondary flight feathers and tail feathers (Fig. 1). They often had full crops, distended abdomens, mottled tan-red livers, and reddened skin.

Multiple tissues were fixed in 10% formalin, tissue homogenates were negatively stained with 2% phosphotungstic acid, and kidney, skin, and brain were fixed in 4% formaldehyde-1% glutaraldehyde and routinely processed for light and electron microscopy, respectively. In addition, samples were collected for bacterial culture and virus isolation.

Although the severity of histological lesions varied among birds, essentially similar changes were present in all. The most consistent finding was cells with karyomegaly. In these cells, the nucleus was enlarged, had margined chromatin, and often contained a large slightly basophilic to amphophilic inclusion. These cells were most prominent in feather follicles, which often were severely affected, although not all follicles were involved. Focal to confluent ballooning degeneration with karyomegaly was prominent in follicular epithelial cells, barb ridges, and cells within the pulp (Fig. 2). Karyomegalic cells also were in scattered foci in renal tubular epithelium, glomeruli, brain (Fig. 3), spleen, liver, myocardium, pancreas, intestine, lung, skeletal muscle, and bone marrow.

In many hepatic periportal zones, there was minimal ne-
crosection with a minimal to moderate number of heterophils. Occasional small foci of coagulation necrosis with heterophils were randomly scattered in the parenchyma. Splenic white pulp was hypercellular with small foci of karyomegalic cells present. The walls of many small arteries and sheathed capillaries also were prominent because of hypertrophy and karyomegaly of the surrounding cells.

In the negatively stained tissue homogenates and thin sections of renal tubular epithelium, virus particles were present in small clusters. On the basis of size and morphology (approximately 40 mm in diameter, nonenveloped icosahedral virions), the particles were identified as papovaviruses.

Five live budgerigars (4 adults and 1 fledgling) in the aviary were tested for antibody to BFD virus by fluorescent antibody serum neutralization. All were seropositive (≥ 1:10), with titers ranging from 1:10 to 1:320. No bacterial pathogen or Chlamydia were isolated from any of the tissues examined, and virus isolation was unsuccessful. Based on the history, clinical findings, necropsy results, and serology, a diagnosis of BFD was made.

In an aviary with BFD, nestling budgerigars (1-3 weeks of age) suffer the highest mortality. A striking finding in these birds is the almost total lack of downy feather development, which is prominent even in budgerigars that survive up to 28 days of age. Normal baby budgerigars are hatched without feathers, but down begins to cover the skin by about 12 days of age; plumage development is complete at a little more than 1 month of age. Although other diseases must be considered, death at 10-28 days of age with a significant lack of feather development should alert the diagnostician to the possibility of BFD. The combination of typical gross necropsy findings (stunted growth, abnormal feather development) and microscopic lesions, including pronounced karyomegaly with intranuclear inclusions, leads to a presumptive diagnosis of BFD. This diagnosis should be substantiated by virus isolation or by identification of the virus via specific immunofluorescence. DNA amplification via the polymerase chain reaction can also be used to detect BFD virus.

The transmission and pathogenesis of BFD have not been fully described. Virus particles have been demonstrated in epithelia of feather follicles, esophagus, crop, lung, and kidney, thus implicating several possible routes of transmission. Feather dust, regurgitated food fed to nestlings, respiratory secretions, and/or urinary excretions could all contain infectious virus and serve as a source of infection. Vertical transmission of the virus through the egg also has been considered. Inclusion bodies have been demonstrated in the internal organs and skin of 1-day-old budgerigars, and eggs from breeding pairs producing affected young will result in diseased birds when fostered by pairs whose own young are normal.

Serologic testing has shown that viral antibodies remain elevated for the lifetime of a persistently infected (carrier) bird. Persistently infected female birds can raise serologically negative uninfected chicks and immunocompetent adult birds can be caged with persistently infected birds and will remain uninfected. The development of immunocompetence is critical. Nestlings unprotected by passive antibody and exposed to papovavirus are susceptible and may die. Those protected may survive but still be contaminated with the virus and serve as a source of infection, which is an important consideration when baby birds are being hand raised and the virus can be easily transmitted.

The economic consequences of BFD in a breeding aviary can be devastating. Although adult birds may be unaffected, the mortality in young birds can be extremely high and can essentially eliminate the successful production and sale of young budgerigars. The year before the outbreak, approximately 200 birds from the aviary in this study were fledged.
whereas only 10 were fledged in the following year. The BFD source was undetermined; however, new adult budgerigars had been introduced into the aviary several months prior to the outbreak, and 1 or more of these birds were probably carriers of the virus. Several other papers have reported mortality rates ranging from 25% to 100% in fledglings; some of these aviaries had up to approximately 1,200 breeders. One report described a decrease in hatchability from 80% to 40% in an aviary with 90 breeding pairs. 

Once BFD is present in an aviary, successful elimination of the disease, short of depopulation, is very difficult. There is no known treatment, and a vaccine is not commercially available. Preventive measures include maintaining a totally closed flock and prohibiting visitors and visitation to other flocks. Control of the disease via thorough disinfection of the aviary using chlorine or phenolic solutions and fumigation with formaldehyde gas has been recommended and was successful in 1 aviary. Interruption of breeding (budgerigars breed year round), thus removing the supply of susceptible birds, has also been recommended but has met with limited success. Successful elimination of BFD from an aviary was achieved via depopulation, decontamination, and restocking with seronegative birds.

Since the initial descriptions of BFD in budgerigars, similar lesions have been described in a variety of other pet bird species, including conures, macaws, Amazon parrots, cockatoos, cockatiels, lorries, lovebirds, parakeets, and finches. Serologic tests have shown that a variety of birds can be infected by papovavirus.

Papovavirus infections in budgerigars are clinically and pathologically different from these infections in other birds. In budgerigars, BFD primarily presents as an acutely fatal disease in birds up to 21 days of age or as a more chronic disease with feather anomalies in older birds. In conures, Amazon parrots, cockatoos, cockatiels, lorries, and macaws, age at the time of death is somewhat older, ranging from nestling to 16 weeks, although death in splendid parakeets, Gouldian finches and lovebirds occurred at 3 and 8 months, as fledglings to immature adults, and at < 1 year old, respectively.

Abnormalities in feather development and structure, lesions typically described in young budgerigars with papovavirus disease, are not commonly seen in other species of pet birds. A variety of gross lesions are described, but all are nonspecific and include pallor of muscles, hepatomegaly with a mottled discoloration, petechia and ecchymoses, splenomegaly, hydropericardium, and ascites.

As in budgerigars, the most consistently observed histologic lesion in other birds is karyomegaly, which occurs in a variety of tissues and often is associated with large intranuclear inclusions. Multifocal hepatic coagulative necrosis is also a consistent and significant lesion, although it is variable in extent. In some cases, liver necrosis is limited to scattered microfoci, although in others the necrosis is more confluent. Although the classic feather anomalies typically seen in budgerigars are not seen in other birds, a presumptive diagnosis...
of papovavirus infection can often be made on the basis of clinical signs; postmortem findings, including light and electron microscopic lesions; and serology.

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**References**


**A comparison of isolation and a commercial ELISA for the diagnosis of chlamydiosis in psittacine birds**

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The problem of identifying birds that are persistently or latently infected with *Chlamydia* and the desire for more rapid diagnosis of diseased animals has resulted in the development of many new test procedures. As advances in technology supply diagnostic laboratories with an increasing variety of tests, each laboratory must choose tests appropriate to their circumstances. Each method attempts to maximize sensitivity and specificity while minimizing cost, time, specialized equipment, and technical expertise required to perform the test. Several recent comparisons of the available techniques for diagnosing Chlamydia infections have not made the choice easy. A commercial enzyme-linked immunosorbent assay (ELISA) was reported to be as sensitive as culture in diagnosing *Chlamydia psittaci* infections in birds submitted for necropsy. The same test kit was reported to be “ideally suited for office use” in cases of human conjunctivitis; although it was “not quite as sensitive as culture,” it was less costly, faster, and easier to perform. Culture and peroxidase-antiperoxidase (PAP) examination of tissues were...