Cryptosporidiosis in four cockatoos with psittacine beak and feather disease

Kenneth S. Latimer, DVM, PhD; W. L. Steffens III, PhD; Pauline M. Rakich, DVM, PhD; Branson W. Ritchie, DVM, MS; Frank D. Niagro, PhD; Ingrid M. Kircher, MS; Phil D. Lukert, DVM, PhD

Two adult citron-crested cockatoos *(Cacatua sulphurea cintrinocristata)*, an adult lesser sulphur-crested cockatoo *(C sulphurea sulphurea)*, and a juvenile umbrella cockatoo *(C alba)* were admitted for evaluation of feather dystrophy and loss. In each instance, the presumptive clinical diagnosis was psittacine beak and feather disease (PBFD). These birds subsequently were donated for further observation and testing.

An adult citron-crested cockatoo (bird 1) of undetermined gender had severe feather dystrophy and loss affecting more than three quarters of its plumage. Beak lesions were not apparent. During hospital observation, the bird developed intermittent diarrhea, but its appetite and attitude were considered normal. Nine weeks later, the bird had an acute onset of severe diarrhea lasting 7 days. Despite treatment for diarrhea, the bird was found dead in its enclosure.

A 1.5-year-old hand-raised female citron-crested cockatoo (bird 2) had severe feather dystrophy and loss of three quarters of its plumage. A 1-year-old sibling had died from PBFD and secondary bacterial infection. In addition to feather abnormalities, bird 2 had intermittent diarrhea during a 1-year observation period. The cockatoo was euthanatized after a 1-week period of diarrhea, anorexia, and signs of depression that were unresponsive to treatment.

An adult female lesser sulphur-crested cockatoo (bird 3) had mild feather dystrophy and loss affecting approximately one fifth of its feathers. The bird died unexpectedly 2 months after presentation, after several days of protracted diarrhea that was unresponsive to treatment.

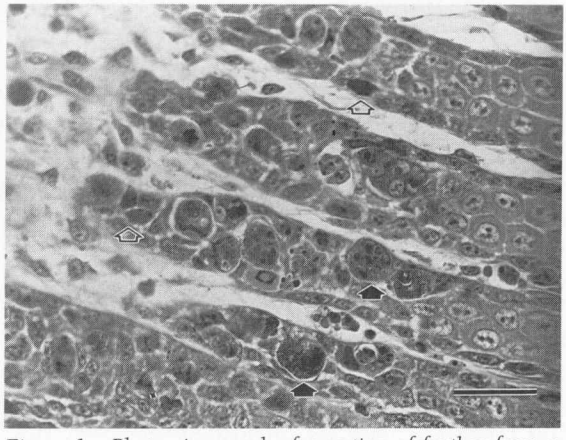

Figure 1—Photomicrograph of a section of feather from a citron-crested cockatoo with psittacine beak and feather disease. Notice (dark) positive staining of macrophage cytoplasmic inclusions (closed arrows) and epithelial cell intranuclear inclusions (open arrows) for psittacine beak and feather disease viral antigen. Avidin-biotin complex immunoperoxidase staining with hematoxylin counterstain; bar = 25 μm.

A 20-week-old female umbrella cockatoo (bird 4) had moderate feather dystrophy and loss affecting 60% of its feathers. The bird died suddenly, 5 weeks after presentation. Mild intermittent diarrhea was observed during the last week of life.

After admission to the hospital, feather follicle biopsies or several plucked feathers were obtained from each bird and submitted in neutral buffered 10% formalin solution for histologic examination. Tissues were processed routinely, embedded in paraffin, sectioned at 4 μm, stained with H&E, and examined microscopically. Replicate tissue sections of paraffin-embedded cutaneous tissues or plucked feathers were stained by the avidin-biotin complex immunoperoxidase technique to demonstrate PBFD viral antigen.[1]

Cutaneous biopsy specimens from all birds had features typical of PBFD, including multifocal necrosis of the feather epithelium, basophilic nuclear inclusions within some epithelial cells, and

From the Departments of Veterinary Pathology (Latimer, Kircher), Anatomy and Radiology (Steffens), Small Animal Medicine (Ritchie), Medical Microbiology (Niagro, Lukert), and the Athens Diagnostic and Investigational Laboratory (Rakich), College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

Supported in part by the University of Georgia Companion Animal Research Fund (grant No. 10-21-RR208-083; CA-036), the Department of Veterinary Pathology, and private contributions by Dr. John Vaughn, Terry Clyne, the International Avian Research Foundation, and Zeigler Brothers Inc.

The authors thank Mary Ard, Rachael Bailey, Kathy Davis, Gwin Kerce, Kim Towson, and Christy Farmer for technical assistance.

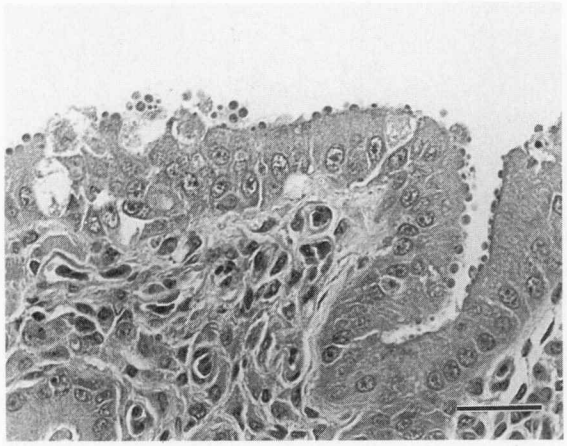

Figure 2—Photomicrograph of section of intestine from a lesser sulphur-crested cockatoo. Notice numerous cryptosporidia embedded in the intestinal brush border. H&E *stain; bar = 25 μm.*

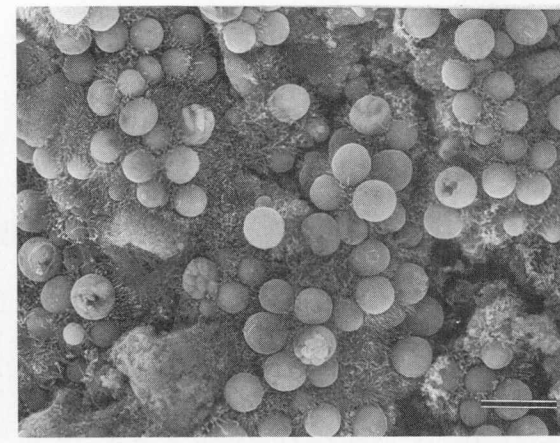

Figure 3—Scanning electron micrograph of section of bursal epithelium of a citron-crested cockatoo. Notice numerous cryptosporidia embedded in the luminal surface. Osmium-thiocarbohydrazide-osmium-ligand osmium binding; bar = 6 μm.

globular basophilic cytoplasmic inclusions within macrophages in the epithelium and feather pulp cavity. Cellular infiltrates within the pulp cavity ranged from mononuclear to heterophilic. Inclusions and necrotic epithelial cells stained positively for PBFD viral antigen by the avidin-biotin complex immunoperoxidase technique (Fig 1). Feather sheaths were hyperplastic. Scattered follicular epithelial cell necrosis was evident in tissues from bird 4. Although a few inclusions were observed within follicular epithelial cell nuclei and macrophages, lesions were less dramatic than those in corresponding feathers.

All birds were necropsied. Portions of all major organs were collected and preserved in neutral buffered 10% formalin solution. Replicate tissue sections were stained by the Brown and Brenn, Giemsa, Macchiavello's, and periodic acid-Schiff techniques as needed to demonstrate infective agents. Tissue sections containing suspected PBFD viral inclusions were stained by the immunoperoxidase technique.[1]

At necropsy, the developing feathers of birds 1, 2, and 4 were visibly clubbed or deformed. Feather sheaths seemed thickened, and some pulp cavities were discolored red-brown to black. In contrast, bird 3 had mild feather dystrophy. Its intestinal tract was dilated with gas and green fluid.

Microscopically, all birds had cryptosporidiosis. In birds 1, 2, and 4, cryptosporidia were localized to the bursal epithelium. Organisms appeared as small round 4- to 5-μm basophilic spheres embedded within the brush border. In bird 3, a heavy population of cryptosporidia was observed throughout the small intestine, large intestine, and bursal epithelium (Fig 2).

In addition, bird 1 had septic purulent peritonitis (gram-positive rods) and erosive mycotic ventriculitis (*Candida* sp). Bursal lymphoid necrosis and cystic change were evident. Bird 2 had multifocal to diffuse hepatic and splenic necrosis. Giemsa and Macchiavello's staining revealed chlamydial elementary bodies. Thymic tissue had heterophilic inflammation and cytoplasmic viral inclusions within macrophages. Bird 3 had hepatic and splenic necrosis secondary to chlamydiosis. Severe bursal lymphoid depletion was detected. Bird 4 had septicemia wherein gram-negative rods were apparent within blood vessels, especially within the myocardium. The medullary portion of the bursa was necrotic and contained numerous purple globular cytoplasmic inclusions within macrophages. Basophilic inclusions within thymic (bird 2) and bursal (bird 4) tissues stained strongly for PBFD viral antigen, using the avidin-biotin complex immunoperoxidase technique.

After microscopic examination of postmortem tissues, additional portions of formalin-fixed intestine and/or bursal tissue were diced into 1-mm cubes and placed in Trump fixative. Tissues for transmission electron microscopy were fixed again in osmium tetroxide, dehydrated in graded alcohols, embedded in Spurr low-viscosity resin, stained with lead citrate and uranyl acetate, and examined at 100 kV. Formalin-fixed specimens for scanning electron microscopy were labeled by the osmium-thiocarbohydrazide-osmium ligand binding technique, dehydrated in a series of graded ethanol to 100% acetone, critical point dried, mounted on aluminum stubs, and viewed.[2]

Transmission electron microscopy on intestinal and/or bursal tissues revealed organisms with typical features of cryptosporidia, including a parasitophorous vacuole, unique attachment zone, and a few merozoites. Bursal inclusions in bird 4 consisted of paracrystalline arrays of viral particles within the cytoplasm of macrophages. Displacement of intestinal microvilli was most apparent on scanning electron microscopy where numerous

protozoa were embedded within the brush border (Fig 3). In bird 3, intestinal microvilli were almost obliterated by organisms.

Cryptosporidiosis is caused by a coccidian parasite that is associated with gastroenteritis and diarrhea in a variety of animal species.[3,4] Although the organism may be a primary pathogen, more severe disease often is observed in individuals with immunodeficiency.[4] Cryptosporidiosis is common in chickens and turkeys, but is uncommon in pet birds.[5] Although auramine O-stained cryptosporidial oocysts have been observed in fecal specimens from a budgerigar, parrot, and macaw,[6] histologic descriptions of intestinal or bursal cryptosporidiosis in pet birds are rare. Intestinal cryptosporidiosis has been reported in a budgerigar and a cockatiel,[7] and involvement of the bursal epithelium has been described in 2 red-lored amazon parrots (*Amazona autumnalis*).[8]

Psittacine beak and feather disease was originally described in the mid-1970s in South Pacific psittacine birds. The disease is commonly characterized by symmetrical feather dystrophy and loss. Beak deformities are less frequent but may be present.[9] Recent evidence indicates that the disease is caused by a novel 14- to 17-nm, icosahedral, nonenveloped virus containing a single-stranded, circular DNA genome.[10]

Clinical evidence suggests that PBFD apparently is associated with acquired immunodeficiency. Viral-associated infection and destruction of the thymus and bursa of Fabricius have been observed histologically.[1,11] In addition, hypogammaglobulinemia has been documented in diseased birds,[12] and birds with clinically apparent PBFD have been shown to have an increased prevalence of secondary bacterial, viral, and fungal infections.[1]

The birds of this report had major lesions of thymic or bursal tissue, which could impair their immune status. Thymic involution or regression may be observed at sexual maturity, followed by periodic hypertrophy after the breeding season in some birds[13]; however, inflammation and inclusion bodies are not a part of normal dynamic changes in this organ. Likewise, the bursa of Fabricius may undergo regression at sexual maturity. This normal involution is associated with decreased numbers of lymphocytes, increased visual prominence of the connective tissue stroma, and cystic change.[13] Normal involution may explain the cystic change and lymphoid depletion in bursal tissue from the citron-crested (bird 1) and lesser sulphur-crested (bird 3) cockatoos, respectively. However, the lymphoid necrosis observed in bursal tissues from birds 1 and 4 usually is associated with viral infection. This assumption was documented in bird 4 by observing bursal inclusions composed of paracrystalline arrays of viral particles similar to those observed within affected feathers of diseased birds.[11,12] Bacterial (birds 1 and 4), chlamydial (birds 2 and 3), and mycotic (bird 1) infections also were apparent.

Acquired immunodeficiency and cryptosporidiosis have been associated with various viral infections, including human immunodeficiency virus,[4,14] infectious bursal disease virus,[15] reovirus,[16,17] turkey viral hepatitis,[18] canine distemper virus,[19] and feline leukemia virus.[20] In addition, cryptosporidiosis has been observed as an incidental finding in swine with concurrent adenovirus infection.[21] Current reviews suggest that infection with *Cryptosporidium* is more severe in immunosuppressed or immunodeficient patients.[3,4]

We speculate that cryptosporidiosis and other secondary infections in these birds were sequelae of PBFD. Although thymic and bursal involution develop in adult birds, lymphoid necrosis, heterophilic inflammation, and inclusion bodies are not typical.[13] Thymic and bursal PBFD inclusions in 2 birds, and visualization of virus in bursal tissue from one of these birds suggested that PBFD infection was responsible for destruction of lymphoid tissue. This speculation is corroborated by more detailed studies of extracutaneous viral inclusions in birds with PBFD.[1] Psittacine beak and feather disease may have a protracted clinical course lasting months to years.[9] Conversely, acute bacterial peritonitis, gram-negative septicemia, and chlamydiosis (with massive splenic and hepatic necrosis) have a clinical course of hours to days. Therefore, we believe that infection with PBFD virus and destruction of lymphoid tissues probably predisposed the cockatoos of this report to cryptosporidiosis and other secondary infections.

1. Latimer KS, Rakich PM, Kircher IM, et al. Extracutaneous viral inclusions in psittacine beak and feather disease. *J Vet Diagn Invest* 1990;2:204–207.

2. Kelley RO, Dekker RAF, Bluemink JG. Ligand-mediated osmium binding: its application in coating biological specimens for scanning electron microscopy. *J Ultrastruct Res* 1973;45:254–258.

3. Current WL. Cryptosporidiosis. *J Am Vet Med Assoc* 1985;187:1334–1338.

4. Fayer R, Ungar BLP. Cryptosporidium spp. and cryptosporidiosis. *Microbiol Rev* 1986;50:458–483.

5. Goodwin MA. Cryptosporidiosis in birds—a review. *Avian Pathol* 1989;18:365–384.

6. Ley DH, Levy MG, Hunter L, et al. Cryptosporidia-positive rates of avian necropsy accessions determined by examination of auramine O-stained fecal smears. *Avian Dis* 1988; 32:108–113.

7. Goodwin MA, Krabill VA. Diarrhea associated with small-intestinal cryptosporidiosis in a budgerigar and in a cockatiel. *Avian Dis* 1989;33:829–833.

8. Doster AR, Mahaffey EA, McClearen JR. Cryptosporidia in the cloacal coprodeum of red-lored parrots (Amazona autumnalis). *Avian Dis* 1979;23:654–661.

9. Ritchie BW, Niagro FD, Lukert PD, et al. A review of psittacine beak and feather disease. Characteristics of the PBFD virus. *J Assoc Avian Vet* 1989;3:143–149.

10. Ritchie BW, Niagro FD, Lukert PD, et al. Characterization of a new virus from cockatoos with psittacine beak and feather disease. *Virology* 1989;171:83–88.

11. Pass DA, Perry RA. The pathology of psittacine beak and feather disease. *Aust Vet J* 1984;61:69–74.

12. Jacobson ER, Clubb S, Simpson C, et al. Feather and beak dystrophy and necrosis in cockatoos: clinicopathologic evaluations. *J Am Vet Med Assoc* 1986;189:999-1005.

13. Riddell C. Avian histopathology. 1st ed. Kennett Square, Pa: American Association of Avian Pathologists, 1987; 8–9.

14. Guarda LA, Stein SA, Cleary KA, et al. Human cryptosporidiosis in the acquired immune deficiency syndrome. *Arch Pathol Lab Med* 1983;107:562–566.

15. Levy MG, Ley DH, Barnes HJ, et al. Experimental cryptosporidiosis and infectious bursal disease virus infection of specific-pathogen-free chickens. *Avian Dis* 1988;32:803–811.

16. Guy JS, Levy MG, Ley DH, et al. Experimental reproduction of enteritis in bobwhite quail (Colinus virginianus) with cryptosporidium and reovirus. *Avian Dis* 1987;31:713–722.

17. Ritter GD, Ley DH, Levy M, et al. Intestinal cryptosporidiosis and reovirus isolation from bobwhite quail (Colinus virginianus) with enteritis. *Avian Dis* 1986;30:603–608.

18. Wages DP, Ficken MD. Cryptosporidiosis and turkey viral hepatitis in turkeys. *Avian Dis* 1989;33:191–194.

19. Turnwald GH, Barta O, Taylor HW, et al. Cryptosporidiosis associated with immunosuppression attributable to distemper in a pup. *J Am Vet Med Assoc* 1988;192:79–81.

20. Monticello TM, Levy MG, Bunch SE, et al. Cryptosporidiosis in a feline leukemia virus-positive cat. *J Am Vet Med Assoc* 1987;191:705–706.

21. Sanford SE. Enteric cryptosporidial infection in pigs: 184 cases (1981-1985). *J Am Vet Med Assoc* 1987;190:695–698.

Book Review: Domestic Animal Behavior for Veterinarians and Animal Scientists, Second Edition

The growing acceptance of companion animals as members of the human family, and the resurgence of animal welfare as a social issue appear to be increasing professional interest in animal behavior. Students want to understand the basis for an animal's behavior to solve behavioral problems that confront pet owners and to assess farm animal behavior as an indicator of animal welfare. The Preface notes that this edition, unlike the first one (1982), deletes chapters addressing the human/animal bond and cruelty to animals because this material is now well developed elsewhere. Although this is somewhat unfortunate, the astute student can use the book's detailed information to better understand behavioral dysfunction and to appreciate welfare issues.

This book is clearly a text book for professionals and most entries of materials are well referenced. There are numerous clear figures and those of the author's own studies are particularly good. The book is organized by system, and the physiologic basis for behavior is well developed, but less attention is given to evolutionary and ecologic principles.

Veterinarians, pet and farm animal practitioners may find that too little attention is paid to behavioral problems in a practical way. Nevertheless, the book is an incredible synthesis of material and a marvelous source of basic information for the dedicated student or researcher in the field.—*[Domestic Animal Behavior for Veterinarians and Animal Scientists, Second Edition. By Katherine Albro Houpt. 416 pages; illustrated. Iowa State University Press, 2121 S State Avenue, Ames, IA 50010. 1991. Price $39.95 ($2.00 shipping).]*—ALAN M. BECK