



**Documentation of a PBFD virus Variant
and its
Importance to PBFD Testing**

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Psittacine beak and feather disease (PBFD) was first described in various species of cockatoos in the early 1970s. The disease initially was characterized by symmetric feather dystrophy and loss, development of beak deformities and eventual death. In the mid 1980s, it was demonstrated that PBFD was caused by a previously undescribed virus that represents one of the prototype viruses for the Circoviridae family of viruses.¹ In addition to psittacine birds, circoviruses have been reported in pigs,²⁻⁵ chickens,⁶ humans,⁷ pigeons,⁸⁻¹⁶ Senegal doves,^{17,18} canaries,¹⁹ finches,²⁰ geese,²¹ a southern black-backed gull,²² an ostrich,²³ cattle and goats. (B. Nordhausen, personal communication)

Research data collected from the past 12 years has suggested that the protein structure and genome of the PBFD virus affecting psittacine birds (a virus we are now calling PBFD virus 1) was relatively conserved.¹ However, a variant of PBFD virus (a virus we are calling PBFD virus 2) has been documented as the cause of feather dystrophy in a group of lories. Sequence analysis confirms that PBFD virus 2 has sufficient nucleic acid differences that it is not detected using the proprietary nucleic acid primers^a to PBFD virus 1 developed by the Psittacine Disease Research Group at the University of Georgia College of Veterinary Medicine.²⁴

The PBFD virus variant was detected when 9 blood and feather samples from lories with dystrophic feathers were examined. DNA in situ hybridization with a virus specific probe demonstrated that cells associated with affected feathers from all of these lories contained circoviral nucleic acid. Using proprietary PBFD virus 1 specific primers, PBFD virus 1 nucleic acid was not detected in the blood of these lories. By comparison, 16 budgerigars with dystrophic feathers confirmed by in situ hybridization to be associated with PBFD virus were also blood positive for PBFD virus 1 nucleic acid.

Sequence analysis of the viral DNA amplified from the tissues of affected lories confirmed that they were infected with a variant (PBFD virus 2) that would not have been amplified with PBFD virus 1 specific primers. It has recently been reported that a PBFD-type virus recovered from lories in Australia had a nucleic acid sequence that also differed from published PBFD virus 1 sequence.^{24,25}

Most birds infected with PBFD virus 1 develop a transient infection that can be detected by



demonstrating target segments of nucleic acid in the blood. The immune response clears the virus in these subclinically infected birds with no detectable affect on the host. The clinical changes that occur in birds that do not mount an effective immune response against PBFD virus 1 can be peracute, acute or chronic. Peracute infection is typically associated with septicemia accompanied by pneumonia, enteritis, rapid weight loss and death. These birds frequently die before feather abnormalities are easily recognized. Birds that survive peracute or acute disease develop the chronic form of disease associated with PBFD virus 1, which is characterized by the symmetric, progressive appearance of abnormally developed feathers during each successive molt.¹

In general, PBFD virus 1 associated disease in Old World psittacine birds is considered progressive and fatal. By comparison, some New World psittacine birds with established PBFD virus 1 associated disease have been shown to recover.¹ The documentation of PBFD virus 2 would be academic were it not for the fact that the microscopic lesions in affected lorries were less severe than those seen with PBFD virus 1 and several of the affected lorries apparently recovered. It is of interest that two porcine circoviruses have been defined; porcine circovirus 1 (PCV 1) and porcine circovirus 2 (PCV 2).³⁻⁵ These viruses have 76% sequence homology yet vary dramatically in pathogenicity. Porcine circovirus 1 is considered of low to no pathogenicity, while PCV 2 causes substantial disease in affected pigs.³⁻⁵

Management of birds with dystrophic feathers in which PBFD virus nucleic acid is detected has been based on data collected by the Psittacine Disease Research Group using proprietary PBFD virus 1 sequences.¹ It should be considered that when several variants of an organism infect a related population of hosts, the specificity of a diagnostic test usually dictates prognosis. Because PBFD virus 2 appears to behave differently in some birds than PBFD virus 1, clinicians must be extremely careful to determine whether nucleic acid detection technology is documenting PBFD virus 1, PBFD virus 2 or some other target sequence that cross-reacts with conserved PBFD virus or circovirus sequences. Use of less specific primers (designed to amplify target sequence that may not be specific to PBFD virus 1) to screen for viral DNA can result in improper recommendations for patients that were test positive for a circovirus, but not PBFD virus 1.

To date, monospecific PBFD virus 2 infections have only been documented in lorries. However, mixed PBFD virus 1 and PBFD virus 2 infections were documented in approximately 30% of affected psittacine birds with mixed infections being most common in lovebirds. These mixed infections may result in a disease progression that is different from that described for PBFD virus 1. Several reports from the mid-80s suggested that some budgerigars, lorikeets and lovebirds recovered from PBFD-like feather abnormalities.^{26,27} Since these reports predate the documentation of PBFD virus as the cause of PBFD, it remains undetermined if these birds



recovered from polyomavirus, some other cause of feather dystrophy or a less pathogenic variant of PBFD virus, like PBFD virus 2.

If a bird infected with PBFD virus 2 has a greater chance of recovery compared to those infected with PBFD virus 1, then euthanasia of a bird with feather abnormalities associated with PBFD virus 2 would be a disservice to the individual patient and its species. It should be stressed that the management of the individual patient is different from that of the flock and diseased birds must be completely separated from others (see Figure 1).²⁸ Birds that are able to recover from PBFD virus 2 associated disease may transfer the factors responsible for their recovery to their chicks which would be a decided genetic advantage for that lineage of birds.

Based on the documentation of a PBFD virus variant, we suggest the following flow chart to replace those previously published for the detection and management of PBFD virus.

Figure 1. Diagnostic flow chart for PBFD virus

Bird Has Normal Feathers

Test blood for PBFD viral nucleic acid using DNA probe-based assay

- A positive test in a bird with no feather abnormalities indicates that the bird has been exposed to PBFD virus and that viral nucleic acid is present in the blood. This bird must be retested in 90 days. If the bird is still positive when retested at 90 days, this indicates that the bird is either subclinically infected or that the bird is being repeatedly exposed to the virus. Subclinically infected birds can develop feather lesions at some future date. If the bird is negative when retested, this indicates that the bird was transiently infected and that the bird's immune system was able to clear the viral nucleic acid from the blood. Birds with normal feathers that have cleared an infection should be considered resistant to PBFD. Most birds that are exposed to the PBFD virus will have viral nucleic acid present in their blood for a brief period.
- A negative test indicates that the target segment of PBFD viral nucleic acid was not detected in the blood.

Bird Has Abnormally Developing Feathers

Submit affected feathers for histologic examination and blood for PBFD virus 1 nucleic acid detection using DNA probe-based assay

- A positive DNA probe test on the blood of a bird with characteristic inclusion bodies in cells of affected feathers suggests that the bird has an active PBFD virus 1 infection.
- If the feather biopsy contains characteristic inclusion bodies but the blood DNA probe test is negative for PBFD virus 1 nucleic acid, then the blood sample should be retested using a less specific circovirus DNA assay.^a Birds that are found to be infected with variants of PBFD virus other than PBFD virus 1 should be isolated, not euthanized, and monitored closely for the development of normal pin feathers that would suggest recovery. Birds that are



recovering from psittacine beak and feather disease will be blood negative for nucleic acid for months before all of the affected feathers (the cells of which will retain PBFD virus until molted) are replaced during the molting process with new uninfected feathers. As long as dystrophic feathers, or their associated dust, are present the bird should be considered infectious irrespective of its blood DNA status.

- It should be noted that some PBFD-infected psittacines of South American descent have spontaneously recovered from the disease.

Management of a positive bird:

If a bird with feather abnormalities from a breeding aviary is found to be positive for PBFD virus 1, PBFD virus 2 or any variant of circovirus, the bird should be removed from the area as quickly as possible. Actively infected birds with feather abnormalities shed large concentrations of virus in their feather dust which can be easily carried to other birds by the wind or on clothes, skin or hair. All areas, supplies and equipment that could be contaminated with feather dust from the infected bird should be repeatedly cleaned and disinfected. Evaluate whether cleaning efforts following an outbreak have been sufficient by DNA probe testing of air ducts, carpets, enclosures or any dusty area.

Foot notes:

a. The proprietary nucleic acid sequences for PBFD virus 1 were developed by the Psittacine Disease Research Group at the University of Georgia College of Veterinary Medicine and have been licensed by the University of Georgia Research Foundation to several diagnostic laboratories in the United States and Europe. Tests based on this specific technology are currently available in the United States through ANTECH, the Comparative Pathology Laboratory at the University of Miami, IDEXX and the Infectious Diseases Laboratory at the University of Georgia College of Veterinary Medicine. In Europe, this specific technology is available through Zoo and Aquatic Veterinary Group in England or Van Haeringen Laboratorium in the Netherlands.

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