A Practitioner’s Guide to Avian Polyomavirus Testing and Disease

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Since its recognition as a pathogen of birds in the early 1980’s¹,²,⁴ avian polyomavirus (APV) has been a continued cause of mortality in caged birds.³,¹¹,²⁶,²⁹ Recent research has provided new information about the biology and pathogenesis of APV.¹⁴,¹⁷,²¹,²²,²³,²⁴,²⁶ Based in part on this information, both serological and polymerase chain reaction (PCR)-based diagnostic assays have been developed.¹⁸,²¹,³⁰ Despite extensive discussion in the literature on the nature of APV infection and disease and the value of these tests for the control of APV, there is still considerable confusion about all aspects of APV infection, disease, and control. The following text contains and addresses the most common questions asked by practitioners regarding APV.

Q. What is the difference between budgerigar fledgling disease virus, papovavirus, and avian polyomavirus?

A. Nothing; these are the same viruses. The first avian polyomavirus was recognized in and isolated from young budgerigars (Melopsittacus undulatus) and therefore was called the budgerigar fledgling disease virus (BFDV).²,⁴ When it was first described, its characteristics placed it in the family Papovaviridae.²,⁴,⁸ The Papovaviridae contains 2 genera, the papillomaviruses and the polyomaviruses. Detailed characterization of BFDV has shown it to be a polyomavirus. The current trend is to call the virus isolates from psittacine birds avian polyomaviruses.¹⁵,¹⁷,²⁸

Q. Is there only one avian polyomavirus, or do different avian polyomaviruses infect different species of birds?

A. Among psittacine species, there appears to be only one APV. In a recent study, selected parts of the DNA code from 20 viruses obtained from 14 species of birds were compared. Small amounts of genetic variation (point mutations) were found in most of the examined viruses. Using these variations, an evolutionary tree depicting the interrelatedness of these viruses was created. Based on this tree, it was shown that identical viruses could infect multiple species of birds, e. g., the same virus was found in a yellow-collared macaw, eclectus parrot, and green-cheeked conure. Additionally, it was shown that even the most genetically divergent viruses could still infect and

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cause disease in the same species of bird.\textsuperscript{25}

The precise relationship between the APV infecting psittacine birds and the polyomavirus or polyomaviruses infecting passerine birds remains unknown. Histologically, nuclear enlargement (karyomegally), a hallmark of APV infection is seen in both groups of birds.\textsuperscript{9,10,13,16} Also, at least one of the finch viruses has a similar antigenic structure to the psittacine APV.\textsuperscript{16} However, until the genetic composition of both viruses can be compared, the interrelatedness of these viruses remains unknown.

Q. Which psittacine species are susceptible to APV infection and disease?

A. While not every parrot species has been examined, it appears that most, if not all psittacine birds are susceptible to APV infection.\textsuperscript{3,11,12,14,19,22,26,30} Infection and disease, however, are not synonymous. When APV is introduced to an aviary or pet store, it spreads widely often infecting the majority of birds.\textsuperscript{3,12,19,23,24,30} Disease occurs only in selected populations of birds, or may be entirely absent. When disease does occur, it is predominates in macaws, conures, eclectus parrots, ring-necked parakeets, budgerigars, and lovebirds.\textsuperscript{23,25,26} Other species, e. g., cockatiels, lories, caiques, and Amazon and hawk-headed parrots, may uncommonly to rarely develop disease.\textsuperscript{11,25,26}

The vast majority of birds that die from APV disease are hand-raised nestlings between 2 weeks and 14 weeks of age.\textsuperscript{3,11,12,23} Even at this age, however, infection often does not always result in disease. In an aviary experiencing APV deaths in eclectus nestlings, no deaths were observed in several species of cockatoos although all the cockatoo nestlings examined had been infected with APV.\textsuperscript{25} In another epizootic of APV disease, 4 nestling macaws and 1 Amazon parrot were lost and yet 3 siblings (2 Amazon parrots and a macaw) housed with these birds remained healthy even though they were also infected. In contrast to hand-raised nonbudgerigar parrots, APV disease in parent raised birds is rare. In fact, in one case, eclectus chicks raised by virus-shedding adults failed to develop any sign of virus infection or disease.\textsuperscript{25}

Q. Are adult birds also at risk for developing APV disease?

A. In adult psittacine birds, APV disease has been described in eclectus parrots,\textsuperscript{22,26} cockatoos, caiques, a painted conure,\textsuperscript{26} and lovebirds.\textsuperscript{20} In the former 4 species, disease in adult birds is uncommon to rare. Lovebirds, eclectus parrots, and cockatoos are also susceptible to psittacine beak and feather disease and concurrent infections with these 2 viruses occur. Increasing evidence suggests that APV-disease in adult birds requires a concurrent infection with the beak and feather disease virus for disease to develop.

Q. What tests are available for detecting inapparent APV infection in psittacine birds, what do the results of these tests mean, and what are their limitations?

A. Serological assays and PCR (or genetic probe)-based tests are available for determining the history of APV infection and current virus shedding. The detection of anti-APV antibody in a bird indicates a previous or current APV infection. The absence of antibody may mean that the bird has never been infected with APV; that infection occurred previously, but that antibody titers have declined below a detectable level; or
that the bird may even be actively shedding virus, but is not producing anti-APV antibody.\textsuperscript{19,25} Therefore, it is important to understand that both seropositive and seronegative birds can be actively shedding virus. Also, it is important to understand that most seropositive birds are not shedding virus.

Interpretation of serology is also species-dependent. In the budgerigar, antibody titers may persist for many years and possibly for life.\textsuperscript{24} In other species, especially, the cockatiel,\textsuperscript{25} antibody titers may fall to undetectable concentrations after several months to a few years.\textsuperscript{30}

PCR-based tests have the primary advantage of being able to detect virus DNA in droppings and cloacal swabs. Therefore, a positive test implies that the bird is currently infected with APV and is actively shedding the virus. This assay, however, is not without limitations. It is an extremely sensitive assay and false positive results can result from contamination of the sample as it is collected. Because of the sensitivity of this assay, the slightest contamination of the sample can result in false positive results.

Recently, a PCR-based diagnostic assay has been developed that detects APV in the blood (Research Associates Laboratory, Milford, Ohio). This test has the promise of being able to detect persistently shedding birds with a single sample. However, it is still in the development phase, and its sensitivity and specificity are still to be determined.

Q. \textbf{Does a negative PCR (genetic probe) test mean that the tested bird is free of APV infection and will not be a source of the virus for other birds?}

A. A short answer to this question is no, but this is a very complex subject. The dynamics of APV infection and subsequent shedding are only beginning to come to light. In most nonbudgerigar parrots, virus shedding is probably short lived, occurring soon after infection and lasting several days to a few weeks.\textsuperscript{19,25} Detectable virus concentrations are not present every day, and 5-7 samples collected every 2nd or 3rd day are sometimes necessary to detect shedding in a small percentage of birds.\textsuperscript{25}

The frequency of virus shedding may also be species-dependent. Viral activity in budgerigars is highest in young birds before breeding age.\textsuperscript{24} In one report, virus prevalence was highest in submitted lovebird samples.\textsuperscript{5} Concurrent infections with beak and feather virus are common in this species and may influence the prevalence of virus shedding in birds infected with both viruses.\textsuperscript{5}

In a small percentage of birds infected with APV, virus shedding continues intermittently for months and possibly years after infection. The frequency and duration of this shedding is not known. In adult APV-infected parrots sampled 2 times at 4-6 month intervals only 26\% of those found shedding at either date were found to be shedding at both collection dates.\textsuperscript{25}

Thus, a single negative PCR-based test does not rule out APV infection or shedding. Multiple samples over an extended time period are necessary to identify persistently or intermittently shedding birds.
Q. What protocol can be used to minimize the risk that APV will be introduced into an aviary?

A. Preventive measures combine prudent management practices and testing. People who are raising large valuable birds must not raise the often infected lovebird, budgerigar, and cockatiel. Also, until proven otherwise, finches should be kept out of the aviary. Baby birds should be handfed only at their aviary of origin. New birds should be obtained from reputable aviaries. Birds from any pet store must be immediately suspect. Quarantine of new birds for as long as is practical is indicated, and introduction of new birds would preferably be done when little or no breeding is occurring. Repeated testing for virus shedding is indicated, but may prove economically unfeasible. Serology may also prove useful. It cannot be used to predict virus shedding or even infection, however, it may suggest whether or not APV was present in the aviary from which the bird originated. Especially in young birds, positive antibody titers suggest recent APV infections.

Q. How can APV-disease be prevented in the pet store?

A. Pet stores are the perfect environment for APV infection and disease. Most pet stores sell lovebirds, cockatiels, and budgerigars, birds generally come from multiple sources, and hygiene is often inadequate. The simplest solution to the pet store problem is to keep susceptible species under 4 months of age out of the store. Birds can still be purchased at a young age, but must be handfed elsewhere by an employee who doesn't work in the store. Less satisfactory alternatives include serologic monitoring of the flocks from which all birds are being obtained. Lovebirds, in particular, should be screened for evidence of APV and beak and feather virus infection.

Q. My client has recently sold birds which subsequently died with APV disease. How can I determine if the infection originated in my client's aviary or after the bird was sold?

A. At this point, this question cannot be answered definitively because the incubation period from exposure to disease is not known. However, serologic testing of other nestling and young birds in the aviary will supply valuable information. If the APV was present in the original aviary, most young birds would be expected to be seropositive.

Q. What can be done in an aviary which has lost birds with APV?

A. In an outbreak situation, the first objective is to stop introducing susceptible birds into the aviary. In other words, breeding should be stopped if at all possible. Secondly, the young of susceptible species should be raised by the parents until they are weaned. Finally, young birds should not be sold to stores or other aviaries until after weaning, and preferably not until repeatedly found negative for virus shedding.
Q. Can aviaries with previous APV problems produce APV-free offspring in subsequent years?

A. Yes. As was discussed earlier, our current knowledge suggests that most birds stop shedding virus within weeks to months after infection. In these aviaries, if suspect birds, e.g., lovebirds, budgerigars, and cockatiels are eliminated and proper hygiene is instituted, seronegative birds can be produced in subsequent seasons. Testing for virus shedding before the onset of breeding may also be beneficial, but is subject to the limitations described above. Serologic testing of a representative sample of the young birds will demonstrate if infection has been prevented.

Q. How do control measures for APV infection in the budgerigar differ from those used in other species?

A. Although the APV virus infecting budgerigars is the same one that infects other species, certain features of the biology of APV in the budgerigar differ from those of other parrot species. In the budgerigar, the disease occurs in young still in the nest. Once established in an aviary, the prevalence of infection reaches 100% of the birds both young and adult. Although seropositive, young birds continue to shed virus until breeding age. In birds used for continuous breeding virus activity decreases dramatically and shedding may stop altogether.

Based on this information, introduction of APV to budgerigar aviaries can be prevented by purchasing only those birds which are seronegative. Also, it has recently been shown, that APV-free offspring can be produced from seropositive adults. To do this, all young birds are eliminated from the aviary. Older, previously breeding birds, are kept as breeding stock. The aviary is cleaned and disinfected, and breeding is reinitiated after 6 months.

Unexpectedly, the prevalence of APV infection in English budgerigars in aviaries with other species of APV seropositive and virus shedding parrots has been found to be low. These data suggest that the English budgerigar may have a natural resistance to APV infection.

Q. Will a vaccine be the final solution for APV in psittacine birds?

A. On this question, the verdict is still out. To date, only a limited study has been reported on vaccine trials in nestling birds. In this study, virus replication appeared to be inhibited by prior vaccination with the inactivated virus. However, significant questions about APV-disease must still be answered before a vaccine can be considered beneficial, or even safe. First, a model will have to be developed in which infection and disease can be consistently produced using a thoroughly characterized virus. To do this the natural route of infection must be documented. It has been suggested that the primary route of infection may be through ingestion of the virus. However, other studies in budgerigars where only able to induce infection through the respiratory system. In other animal models, the route of infection plays an important role in the pathogenesis of polyomavirus infections.

It will also be necessary to determine if anti-APV antibody is sufficient to prevent
infection. In an outbreak of APV disease in adult eclectus parrots, high antibody titers were detected in 3 birds 2 weeks before they died with APV-disease. A second factor which must also be considered is that neutralizing antibody may actually play a role in the development of APV disease. Recently it has been suggested that the lesions seen in APV-infected nonbudgerigar parrots are immune mediated, requiring the development of antibody before they can occur.\textsuperscript{23}

At this point, I only recommend the use of the vaccine in birds less than 14 weeks that are being shipped to pet stores and other high risk environments. If APV has been a problem in an aviary than the vaccine needs to be administered to birds beginning at 3 weeks of age (Remember, conures first start dying with this disease at 3 weeks.) I feel that the vaccine will do little good in the face of an outbreak, as most birds are already exposed by the time a diagnosis is made. However, most practitioners will vaccinate in this situation in any case in the hopes that it will do some good.

**CONCLUSION**

Although avian polyomavirus may never be eliminated entirely, many means of control are already at hand. Practitioners can play a vital role in preventing this disease and limiting its effects, through client education, introduction of specific management techniques, and the judicious use of serologic and PCR-based testing.

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