

# Avian Polyomavirus: My Thoughts...

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## Introduction

The avian polyomavirus (APV) is one of the most significant viral pathogens of cage birds. It results in substantial economic losses for aviculturists and pet store owners each year. The biology of this virus is complex and as a result veterinarians and aviculturists alike are often very confused about how to best prevent this virus infection and, once confronted with it, how to minimize its impact. The following article is an in depth discussion on APV from my perspective. In it I address issues surrounding the value of testing, what the various tests mean, vaccination, and the various factors that must be understood if the effects of APV are to be mitigated. I feel that this article is timely, as our knowledge of biology and behavior of this virus has grown significantly in the past few years.

## Avian Polyomavirus: A Definition and History

The avian polyomavirus was first recognized in the early 1980s in the southeastern and southcentral United States<sup>4, 9, 10</sup> and in Ontario, Canada in budgerigars.<sup>2, 3</sup> It was called the Budgerigar Fledgling Disease Virus. It was found to be a nonenveloped, DNA virus and based on its size, shape, and DNA content it was classified as a papovavirus.<sup>2, 3, 4, 9, 10, 49</sup> The Papovaviridae contain two very different virus families, the papillomaviruses and the polyomaviruses. With further investigation, it was determined that the Budgerigar Fledgling Disease Virus is a polyomavirus.<sup>26, 29, 57</sup> Subsequently, the virus was found to infect many different species of psittacine birds (parrots) and thus it is generally the convention to call it the avian poly-

omavirus (APV).<sup>5, 17, 20, 35, 45</sup> APV is wide spread and can be found in most countries of the world where psittacine birds are raised.<sup>29, 31, 32, 59, 61</sup> As will become clearly apparent, generalizations about this virus cannot be made and over simplification about the issues of infection and disease, while convenient, are often misleading.

## Avian Polyomavirus Disease Budgerigars

In the Budgerigar, disease and death is confined to nestlings between 10 and 25 days of age.<sup>2, 3, 4, 9</sup> Budgerigar breeders first detect this problem in their flocks when there is a sudden increase in the number of dead nestlings in the nest boxes. The signs of APV disease in Budgerigar nestlings are somewhat variable. Most often, the young birds experience an abbreviated course of disease. At death, birds are found to be stunted, to have abnormal feather development, skin discoloration, abdominal distension, ascites (fluid in the abdomen), enlargement of the liver with localized areas of necrosis (cell death), and scattered areas of hemorrhage. In some outbreaks, the virus attacks the cerebellum (a portion of the brain) and these birds will show head tremors. Microscopic examination of the tissues from these birds reveals virus inclusion bodies in cells of multiple organ systems, including the liver, spleen, kidney, feather follicles, skin, esophagus, brain, and heart.<sup>2, 3, 4, 9, 10, 23, 29</sup>

Not every Budgerigar infected with APV will die. Some survivors will never become outwardly ill and will show no signs of infection. Other nestlings will fail to develop their primary and secondary wing feathers and/or their tail feathers.<sup>3, 18, 22</sup> These

birds have been referred to as runners or creepers and this form of the disease has been described as French molt. It is extremely important to note that another virus, the Psittacine Beak and Feather Disease Virus (PBFDV), can also cause similar signs. It is possible that one or more additional diseases may also cause feather disease in nestling Budgerigars.

Not all Budgerigars appear to be equally susceptible to infection and disease. In one study in the United States, English Budgerigars were rarely found to be infected with APV although they were housed with other birds shedding the virus.<sup>44</sup>

## Nonbudgerigar Parrots

Nonbudgerigar parrots are also susceptible to avian polyomavirus infection.<sup>5, 17, 20, 32, 33, 53</sup> Some are highly susceptible to disease, while others rarely if ever develop disease (Table 1).<sup>37</sup> APV-disease in these birds occurs at different ages in different birds (Fig. 1). In conures, deaths typically occur in birds less than six weeks of age. Deaths in macaws and Eclectus Parrots occur in birds 14 weeks and younger. Most, possibly all, of the nestlings lost are being hand fed.<sup>5, 17, 20, 39</sup> Infected nestlings appear healthy, show very few premonitory signs, and then die suddenly. When signs do occur, they proceed death by only a few hours. Observant owners may notice delayed crop emptying, weakness, a generalized pallor, or bruising under the skin in the preceding hours before death. Yellow discoloration of the urates is another rare observation.<sup>5, 17, 20</sup> Dr. Susan Clubb was able to predict which birds would die, up to 24 hours before their death, by pulling out growing feathers. Birds developing disease would bleed extensively from the feather follicle.<sup>5</sup>

Necropsy findings commonly include generalized pallor with subcutaneous and subsesal hemorrhage and enlargement of the spleen and liver. Less commonly, acites and pericardial effusion may be present. Microscopic examination of the tissues reveals extensive areas of necrosis (cell death) in the liver. Virus inclusion bodies are found in the spleen, mesangial cells of the kidney, and Kupffer cells of

the liver. Necrosis of splenic cells is often massive. Less commonly, virus inclusions are found in other organ systems including the feather follicles.<sup>5, 17, 20</sup> An immune complex glomerulopathy occurs in a significant percentage of the birds with this disease. These complexes contain antibody and APV proteins.<sup>36, 43</sup>

### Lovebirds

APV disease in lovebirds is distinct enough to merit special attention. Like the Budgerigar, this disease occurs in nestling birds and inclusion bodies can be found in multiple organs. Unlike the Budgerigar, birds up to one year of age can also be affected.<sup>32</sup> This unusual age susceptibility has not been fully explained. However, in at least some of these older birds, concurrent infection with PBFVDV is also occurring and may permit APV disease in a bird that would otherwise be resistant to it.<sup>34, 41</sup>

### Infection Versus Disease

It has become evident that infection and disease are not synonymous. Many birds are infected by the virus but only a certain, and sometimes small, percentage of these birds will develop disease.<sup>6, 7, 30, 44, 48</sup> Whether disease will develop is dependent on the species of bird infected, the age of the bird infected, whether that bird is also infected with the PBFVDV, and other factors that remain unclear. Birds that are infected and do not develop disease still have virus replication within their bodies and shed virus in their droppings for a period of time.<sup>6, 7, 30, 44, 48</sup> The length of time that virus shedding occurs, again, depends on the age of the bird at the time of infection and the species of the bird.<sup>6, 7, 48</sup>

### Infection in Budgerigars

As previously mentioned, in the United States, the English variety of Budgerigar appears to have some resistance to APV infection.<sup>44</sup> The most devastating outbreaks of disease occur in large commercial aviaries of the American variety of Budgerigar where birds are bred in rooms containing tens or hundreds of free-flighted birds. Both nestling and adult Budgerigars are susceptible to infection. Death, however, is confined to young birds

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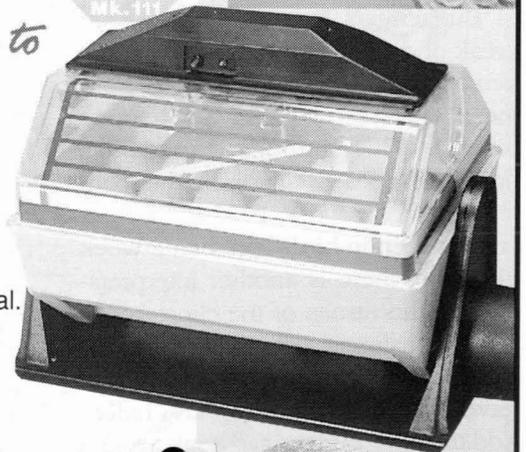
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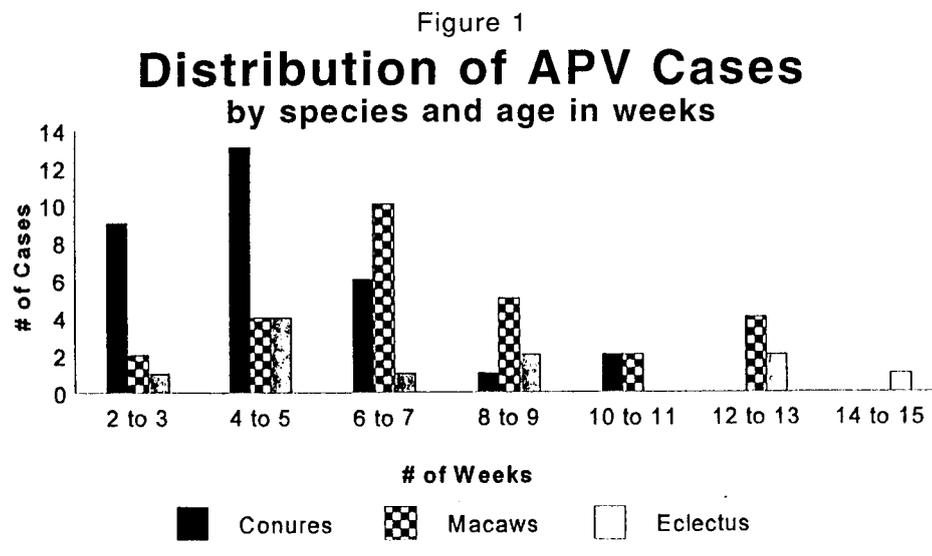
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between the ages of 10 and 25 days. The nestling mortality (death) rate is often high and may approach 100% when the virus is first introduced to an aviary. If there is no intervention in subsequent breeding seasons, mortality rates will decline but production will always remain depressed.<sup>34, 37, 38</sup>

Birds that survive infection may have abnormal feathering or appear completely healthy.<sup>18, 32, 38</sup> Survivors shed virus in their droppings and probably from their skin and feather dander for up to six months after infection.<sup>33</sup> Virus shedding stops with the onset of sexual maturity or during the first breeding cycle. The infection cycle is then maintained through the shedding of virus by nestlings and young adult birds. Thus birds are exposed to the virus immediately after hatch and have virus circulating in their blood by the time they are seven to 10 days old.<sup>37</sup> Fledglings and young adult birds are also important sources of virus exposure for other birds when they are taken to bird shows, bird marts, and sold to pet stores.

It has been suggested that egg transmission of APV occurs in the Budgerigar.<sup>10, 56</sup> This conclusion is based on two observations. First, intra-nuclear inclusion bodies were reported in day-old nestlings suggesting that these birds had virus growing in them before they hatched.<sup>2</sup> Secondly, in a clinical trial, eggs were removed from a flock of Budgerigars experiencing an outbreak of disease and placed under the hens of a clean flock. The young from these eggs subsequently developed disease. This author's experience, however, does not support this conclusion. In my work, inclusion bodies are not seen in birds less than a week old. Also, there is another interpretation for the results of the clinical trial. If the transferred eggs were contaminated with virus, then the chicks could have been exposed at hatch. Additionally, Budgerigar hens eat the egg shells. Thus they could have become infected and then passed the infection on to their young. In a paper I presented in Utrecht, The Netherlands, I found very low concentrations of APV DNA in some embryos and very young nestling budgerigars.<sup>37</sup> This data has also been used to suggest that egg



transmission occurs.<sup>56</sup> These birds never developed disease and, subsequently, I found that one of the reagents used in this work was contaminated with viral DNA. Therefore, at this point in my understanding of APV disease in the Budgerigar and other species, I feel that there is only very limited and circumstantial evidence that egg transmission occurs in any parrot species.

Using my data as proof,<sup>38</sup> it has been stated that Budgerigars are the only bird that is continuously infected with APV and remain sources of virus for life.<sup>56</sup> This was not my conclusion, however, and a subsequent paper of mine argues strongly against this conclusion.<sup>42</sup> In the first publication,<sup>38</sup> I found that virus DNA could be detected in tissues of Budgerigars up to four years of age. Virus concentrations were highest in six-month-old birds, but diminished in birds breeding for four months and were even lower in birds continuously breeding for 17 months. Although virus DNA was found in birds of all ages, it was not clear that the older experienced breeding birds were actually shedding virus. In my second study,<sup>42</sup> I took older breeding birds that we knew had been infected with virus and rested them from breeding for seven months. These birds were then allowed to breed and their young were monitored for signs of infection and the development of antibody to the virus (an indication of infection). None of the 107 young birds produced by these previously infected Budgerigars developed disease. Therefore, we must conclude

that older experienced Budgerigar breeders are not sources of virus infection and even if small concentrations of virus DNA can be found in their bodies, they do not actively shed the virus.

#### Infection in Nonbudgerigar Parrots

Susceptible birds infected with APV infection will die. Rarely, a susceptible bird will have transient signs and survive.<sup>5</sup> In birds resistant to disease, infection is unapparent. In these birds, viral DNA can be first detected in blood after which it is detected in the cloaca.<sup>7, 8, 48</sup> Cloacal samples may intermittently be negative, but generally the blood will remain positive.<sup>7, 8, 48</sup> When the bird is about to stop shedding, the blood will become negative and within a week or two, cloacal swabs will also become negative.<sup>48</sup> The length of time that birds are blood and cloacal positive is dependent on the species of bird and the age that it was infected. It appears, for the most part, that the older the bird is at the time of infection, the shorter the duration of shedding.<sup>7, 8, 48</sup>

**Conures:** Many, possibly most, conure nestlings exposed to APV at six weeks of age or younger will develop disease and die. In birds older than six weeks, APV causes an unapparent infection (Fig. 1). In conures, unapparent infections are best detected by examining the blood for virus DNA. Virus shedding can be expected for four to eight weeks in most birds, but up to 16 weeks in the rare individual.<sup>7, 8</sup>

**Macaws:** Macaws are susceptible to APV infection and disease up to approximately 14 weeks of age, after which infection is unapparent. Peak mortality in macaws occurs from four to eight weeks of age (Fig. 1). Unapparently infected birds will become blood positive and cloaca positive. In a recently completed study, two Blue and Gold nestlings that survived infection shed virus 14 weeks. Two fledgling Red-fronted Macaws shed virus for 10 weeks. Adult Blue and Gold Macaws and Hyacinth Macaws shed for six weeks or less. Unlike most other birds examined, the Blue and Gold nestlings were occasionally blood negative, but cloacal positive.<sup>48</sup>

**Eclectus Parrots:** Infection of otherwise healthy nestling Eclectus Parrots will cause their death if they are less than 14 weeks old. Specific studies on the length of virus shedding in these birds have not been done.<sup>39</sup>

**Cockatoos:** As a general statement, cockatoos of any age are highly susceptible to infection with APV, but are extremely resistant to disease. Healthy adult cockatoos are not expected to ever develop APV disease and the same is true for nestling cockatoos under most circumstances. In a recent study, it was found that Goffin's and Ducorp's nestlings exposed to the virus at less than three weeks of age developed abnormal feathers. These birds showed transient signs of a systemic illness, then recovered with supportive care. Older birds and other cockatoo species remained healthy, although nearly all of them became infected.<sup>48</sup> Virus shedding, as determined by cloacal swab, lasted eight to 10 weeks. Virus could be detected in the blood consistently until just before shedding stopped. In this group of birds, cloacal swabs were not consistently positive and several birds that were originally cloacal positive became negative and then positive again.<sup>48</sup>

I have documented two cases of APV disease in nestling cockatoos that resulted in their deaths. Both birds were also infected with the PBFVDV.<sup>47</sup>

### APV Infection and Disease in Adult Parrots; the PBFVDV Connection

APV readily infects adult parrots.<sup>30, 36, 44, 48</sup> Most infections, probably greater than 99.9% of them, are completely asymptomatic. These birds become infected, shed virus for a period of time, and never look or act ill. APV disease, however, has been documented in adult birds.<sup>24, 25, 46, 50, 56</sup> So why do these few adult birds develop APV disease? The answer in most cases is that they are immunosuppressed with a concurrent infection of PBFVDV.<sup>24, 25, 48</sup> The author has documented an outbreak of APV disease in adult Eclectus Parrots.<sup>46</sup> All birds had PBFVDV. Disease has also been identified in adult cockatoos,<sup>24, 25, 34, 58</sup> again when these birds were tested for PBFVDV they have been found to be positive. I have previously mentioned that young adult lovebirds can die with APV disease. Again, concurrent infections with PBFVDV may be the explanation for why. In the author's experience, on every occasion that APV outbreaks have occurred in lovebirds, PBFVDV could also be found in the aviary.

PBFVDV-infected birds are a common source for APV in an aviary.<sup>44</sup> PBFVDV-infected birds, like AIDS patients, have a poorly functioning immune system. Therefore, if they become infected with APV they cannot clear the virus. Some of these birds will develop full blown APV disease and die. Most will become persistently infected. These persistently infected birds will then shed virus continuously from their skin and in their feather dander. This constant virus shedding contaminates the environment and makes it likely that it will be tracked into the nursery.

### Are Caiques and Eclectus Parrots More Susceptible to APV Infection, Even as Adults, Than Other Parrots?

One of the first reports of APV disease in adult birds documented an outbreak where an Eclectus, a Painted Conure, and three White-bellied Caiques died.<sup>50</sup> These birds clearly had APV-disease. They were not, however, tested for the PBFVDV. So we do not

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know if this means that they were normal birds that have a predilection for APV disease, or were birds infected with PBFVDV and were immunosuppressed. Since that time, the author has heard of a number of deaths in adult caiques. However, none of these birds were tested for PBFVDV. Thus, the answer to this question remains elusive and requires further investigation. Because PBFVDV may not cause histologic evidence of disease, the author feels that it is essential that when APV disease occurs in adult birds or in species where it is not normally a problem, that they be tested by DNA probes for the PBFVDV.

I have documented numerous asymptomatic APV infections in adult Eclectus Parrots. Yet, recently I became aware of four adult Eclectus Parrots, originating from one source, that died with APV-disease. These birds were tested negative for PBFVDV. What does this mean? Was this a particularly hot strain of APV or was there another factor involved? At this point we do not know.

### Testing

Currently there are three types of tests available for detecting APV infection in birds; serology, examination of blood for virus DNA, and examination of cloacal swabs for virus DNA.

### Serology

Serology is the examination of the liquid portion of blood (plasma or serum) for antibodies that are made specifically against a virus, bacteria, or fungus. If a bird is infected with APV and survives, it will develop antibody

to the virus.<sup>14, 38, 44, 62</sup> Antibody can be detected in the Budgerigar by nine days after infection, in most other birds antibody is not present in the blood until two to three weeks after infection.<sup>37</sup> Antibody concentrations rise very quickly and by four to six weeks after infection they reach their peak.<sup>48</sup> Antibody to APV can be detected in the blood for months to many years after infection depending on the species.<sup>38, 44</sup> Budgerigars maintain an antibody titer for life.<sup>38</sup> Cockatiels probably only maintain antibody titers for about six months.<sup>44</sup> However, for most parrot species, antibody can be detected for at least two to three years following infection and possibly for many more years.<sup>44</sup> Recently, I tested seven lovebirds, two Eclectus Parrots, one Ring-necked Parakeet, and one Blue-fronted Amazon for anti-APV antibodies. All birds were seropositive five years or more ago and all birds were still positive with high levels of antibody at this testing.

So what does APV serology tell us? In the Budgerigar, it tells us that the bird was infected with APV. If the bird is a young adult it is probably still shedding virus. If the bird is an older experienced breeder it is not shedding virus and most likely will not. A positive antibody titer in a Cockatiel means that the Cockatiel has been infected within the last six months and this bird may be shedding virus. In other parrots, it tells us very little. If the bird has antibody, then we know that it has been infected with virus but we do not know whether the bird is shedding virus. If the bird was infected recently, then it probably *is* shedding virus. If

the bird was infected over 16 weeks ago, then it is probably *not* shedding virus, unless it is also infected with PBFVDV.

Therefore, with the exception of the Budgerigar, serology is generally not very helpful in detecting virus shedding birds. Unfortunately, this test has been inappropriately used in the past. The author is aware of people who have killed or given away their seropositive birds without understanding that they were not necessarily shedding virus.

### PCR Assay of Cloacal Swabs and Blood

The polymerase chain reaction, or PCR, is an assay that has become an incredibly important tool for the diagnosis and control of infectious diseases. This assay takes a low concentration of the APV DNA and amplifies it to a concentration that can be detected. Therefore, as few as 10 copies of the virus can be detected if the test is properly performed.<sup>35</sup> The sensitivity of this test is one of its greatest strengths as well as one of its greatest weaknesses. The potential problem with this assay is that even the smallest contamination of the sample, either at the collection site or in the laboratory will result in a negative sample becoming positive. Therefore, if one is testing multiple birds, it becomes very easy to get the sample from a negative bird contaminated with the feather dust or dried feces from a positive bird.<sup>48</sup>

**Which PCR assay is better?** The original discovery that APV could be detected in the live bird was made by Dr. Frank Niagro at the University of Georgia.<sup>30</sup> He and his collaborators found that APV could be detected in cloacal swabs of unapparently infected birds. This technology was licensed to the Research Associate Laboratories (Drs. Dalhausen and Radabaugh) and has been offered by them for five years. During this time, these scientists have modified and improved this assay and have discovered that APV DNA can also be detected in the blood of birds recently infected with APV.<sup>6, 7</sup>

The bottom line is that neither test will pick up all APV infected birds that are shedding or will be shedding virus.

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Table 1

## Relative Species Susceptibility to APV Disease: Psittacine birds

### Highly Susceptible

Macaws	Conures	Eclectus Parrots
Budgerigars	Lovebirds	Ring-necked parakeets
Caiques		

### Infrequently Reported with Disease

Cockatiels	Lories	Amazon parrots
Hawk-headed parrot	Cockatiels	

### Disease is Rarely or Never Seen

Cockatoos	Quaker parrots	African Grey Parrots
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On the other hand both tests will pick up most birds shedding virus. So which one will you choose to screen birds? In a recently completed study, the cloacal virus PCR, blood PCR and serologic assay were compared.<sup>48</sup> Of all the birds (> 50) that were examined with multiple tests, both tests picked up all but one of the birds that seroconverted. Not all birds were positive on both tests each time. In cockatoos and conures it was found that birds stayed consistently positive with the blood PCR, while several were intermittently positive on the cloacal swab. As virus was cleared from the bird, the blood test became negative first and the cloacal swab became negative to two to four weeks later. Therefore, for these species, I recommend that the blood PCR be used as a screening tool. If it is positive, the bird should be retested in two to three months, if negative, the bird should be quarantined for four additional weeks and then will be considered free of virus shedding.

In macaws, we found that in most situations both tests were positive. Virus shedding and viremia stopped almost simultaneously. In a few rare occasions, however, the blood PCR test was negative when the cloacal swab was positive. Thus in the limited macaw species examined, a cloacal swab may be more sensitive than the blood PCR. In the future, Research Associates may offer a PCR assay that

screens both blood and cloacal samples from the same bird in the same reaction. This should be the most sensitive assay of all.<sup>8</sup>

It has been said that blood PCR testing of live birds following vaccination or swabs of tissue in recently vaccinated birds that die, will detect fragments of DNA from the vaccine. Recent work by Drs. Dalhausen and Radabaugh has shown that viral DNA is never present in the blood of nestlings vaccinated for APV. The veterinarian and bird owner must therefore conclude that if a bird is blood PCR positive, vaccinated or not, that it is infected with APV and is most likely shedding virus.<sup>8</sup>

### The APV Vaccine: Possibly a Tool, Not a Panacea

In the past two years an APV vaccine (Biomune, Lenexa, KS) has been

on the market. The developers of this vaccine are advocating its use in essentially all parrots, and suggest that if adequate numbers of birds are vaccinated that we can essentially eliminate APV as a problem.<sup>1</sup> Not all investigators, however, share this opinion.<sup>15</sup> While we all share in the desire to control this terrible virus, I believe that the widespread and indiscriminate immunization of pet and aviary birds, is often unnecessary and for the aviculturist may not make economic sense.

In somewhat reverse order, consider the following two points. First, if the vaccine is effective, which birds can it be expected to protect from infection and disease? Secondly, do we have significant and substantial data to suggest that the vaccine does work?

### APV immunization to protect from disease

**Adults.** If our goal is to prevent APV disease by immunization, then it is essential to understand basic APV biology. As has been discussed, healthy adult parrots rarely if ever develop disease. Thus, vaccinating adult birds to protect them from APV disease in most cases is unnecessary (see discussion on caiques and Eclectus Parrots).

**Nestlings.** It is the nestling that when infected with APV will die. Recall, however, that only certain nestlings of certain species are susceptible to disease. To protect these nestlings, according to the vaccination manufacturer, nestlings should be vaccinated at five weeks or older and then again two to three weeks later.<sup>1</sup> They

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are said to be protected four weeks after the first immunization. Thus the vaccine has the potential to protect susceptible chicks from infection and disease in the window of nine to 14 weeks.

A review of Figure 1 demonstrates that we cannot immunized conures at an early enough age to protect them. The same is also true for most macaw and Eclectus chicks. Therefore, APV immunization cannot protect most nestlings from infection and death if they are exposed to the virus before the age of nine weeks. For macaws and Eclectus Parrots raised in a virus-free environment, then moved to a high risk environment at nine weeks of age, immunization, if truly efficacious may provide them with protection against infection.

The question then arises, can we immunize adult birds so that they will pass on antibody through the egg yolk and protect their young from infection? This is a valid and important question that does not have a complete answer. In the Budgerigar, antibody positive parents still have young that develop disease. It has been shown, in this species of parrot, that antibody is trans-

ferred to the egg but does not reach the chicks circulation.<sup>40</sup>

We do not know if other parrots transfer antibody to their chicks through the egg. If they do, several points need to be considered. First, antibody concentrations in adult blood have to be high enough to result in a significant concentrations of antibody being incorporated into the yolk. Therefore, adult birds would have to be immunized close to the onset of breeding season every year. Any disruption of breeding birds at this time can be expected to have some negative consequences.

A second point that needs to be considered is that we do not know if antibody alone will protect from infection. However, if we assume that it does and antibody is transferred to the chick through the egg, then the APV vaccine may conceivably protect chicks for approximately five weeks after hatch.

**Immunization to protect from infection**

In adult birds and many nestlings, APV infection is asymptomatic. Yet these asymptotically infected birds shed virus and can cause the virus to spread into the aviary and the nursery. Therefore, birds that are taken off your property, exposed to other birds, and then returned to the property, may benefit from the APV vaccine. To properly protect them, they must be vaccinated twice, beginning at least four weeks before exposure to other birds. Bird marts, bird shows, and bird club meetings are all potential venues for APV transmission to occur. Remember, a bird shedding polyomavirus may look completely healthy.

**Immunization of currently infected or previously infected birds**

Early in our understanding of APV, it was suggested that birds that were shedding virus were incapable of mounting an appropriate immune response.<sup>30</sup> It was then suggested that immunization would cause these birds to stop shedding virus. Today we know differently. All the evidence shows that once infected with APV, birds rapidly produce high concentrations of antibody.<sup>48</sup> Thus, immunizing a

bird actively infected with APV will do nothing.

**Will the APV vaccine protect against infection and disease?**

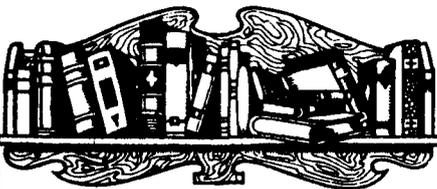
This is an extremely important question that has yet to be answered adequately to my satisfaction. What do we know? We know that several experimental vaccines were successful in inducing a strong antibody response in previously infected birds.<sup>51, 52, 53</sup> However, in birds that did not have evidence of a previous infection, the antibody response to vaccination was minimal.<sup>52</sup>

In another trial, an experimental vaccine was shown to induce a relatively strong antibody response in antibody negative birds.<sup>54</sup> It should also be pointed out, however, that all these birds were in collections where APV had been active previously. As has been discussed, the absence of antibody does not rule out the possibility of previous infection. Thus, many of these birds could have been previously infected and the observed response to the vaccine may have been anamnestic and not primary.

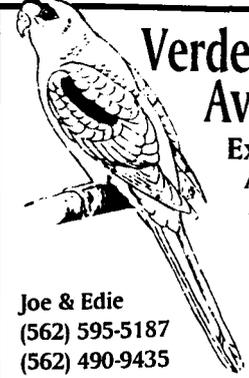
Recently, another study has been published reporting the ability of vaccine to induce an antibody response in birds. In this study, greater than 90% of the immunized birds seroconverted. A placebo group, however, was not included in this study, so it was not proved to my satisfaction that all of the birds that seroconverted were responding to the vaccine, instead of mounting an immune response to a natural APV infection. The design of this study was also very odd in that all immunized birds were greater than 14 weeks old.<sup>56</sup>

Because of the questions that I had with the above studies, I have undertaken my own study of the APV vaccine. Three groups of birds were immunized; seropositive adult birds, seronegative adult birds, and Five-week-old nestlings. The results are as follows:

1. The vaccine had little or no influence on antibody titers in seropositive birds.
2. Adult birds responded to the vaccine by producing antibody. The antibody titers were significantly lower than those seen in natural infections.



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3. Nestling birds immunized at five and seven weeks of age did not develop neutralizing antibody.

What does this mean? My conclusions are that in adult birds with a substantial antibody titer, that immunization is not beneficial.<sup>28a</sup> This is consistent with data on other vaccines in other animals. Immunizing an adult bird with a low antibody titer may be beneficial, but I was unable to find these birds for my study. Immunizing naive adult birds does induce an antibody titer, but the induced antibody titer is relatively low as compared to that of a natural infection. Is the antibody titer achieved by the vaccine sufficient to protect from infection? We do not know. What we do know is that, in dogs, protection against the canine distemper virus and the canine parovirus require that serum antibody must reach a certain concentration.<sup>28a</sup> Experimental infection trials in adult birds using the natural route of infection will be necessary to prove that the vaccine is truly effective.

To protect against a virus infection the body uses both antibody and T cells. Depending on the vaccine, the humoral (antibody mediated) and/or T cell immune response can be induced. Most killed vaccines, however, only induce a humoral immune response. We have shown that several species of nestling parrots do not produce antibody when vaccinated at five and seven weeks of age. Does this mean that the vaccine does not work? Again, the answer is that I do not know. It is possible that it may induce a T cell response. However, until better studies are done, we must err on the side of caution and assume that it does not. At this point in time, I do not believe that we have sufficient evidence to believe that nestlings immunized at this age are protected by the vaccine.

### Disease Prevention

#### *The Nonbudgerigar Aviary*

Each aviary will be unique in its composition of birds and management. But disease prevention will always depend on a balance of testing,

the use of quarantine, and common sense management techniques.

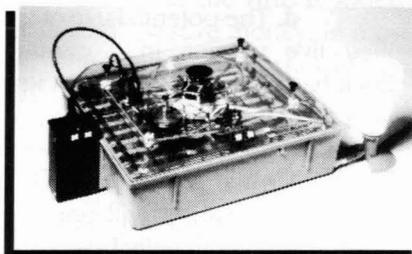
1. In aviaries where the larger parrot species are being raised. The aviculturist should be encouraged not to keep and breed Budgerigars, lovebirds, and Cockatiels. If these species are to be kept, each of these birds should be tested for APV infection. Budgerigars can be tested by serology, lovebirds and Cockatiels by blood PCR.

2. Aviculturists should be strongly encouraged to raise only their own babies and not bring babies from other sources onto their property.

3. Ideally, birds should not be moved off the aviary, exposed to other birds, and returned to the aviary. If this is going to be done, then the returning birds must be quarantined and tested.

4. If birds are going to be moved out of and then back into the aviary. They must be 18 weeks old or older and vaccinated twice — at four weeks and two weeks before they leave the aviary.

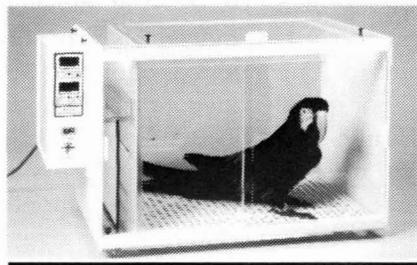
5. Traffic control in the aviary should be such that APV has a limited



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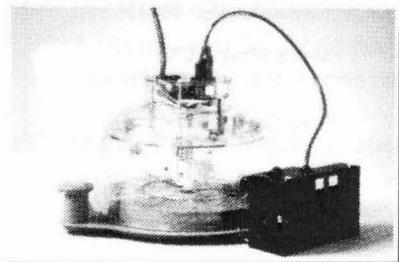
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chance of movement from adult birds to nestlings.

6. All new birds entering the aviary must be quarantined and tested for APV by PCR before they are put in with the breeding birds. Appropriate species should also be tested for PBFDV.

Should all adult birds on the aviary be immunized? This is an important and difficult question. In the author's experience, if APV has previously been present in the aviary, most adult birds (60-90%) will have been previously infected and are naturally immune.<sup>38 44 48</sup> Immunization of these birds would be of little benefit. If APV has not been present in the aviary, then an immunization program might be of benefit if the aviary is at high risk for exposure.

Should all adult birds in the aviary be tested for APV infection? In an ideal situation where money was not a factor, the answer would be yes. In addition, appropriate species should also be tested for PBFDV. PBFDV infected birds will shed both PBFDV and APV continually.<sup>41</sup> Thus, testing for PBFDV in the appropriate species will eliminate both the threat of PBFDV and reduce the threat of APV.

In general, virus shedding in birds other than Budgerigars and Cockatiels lasts less than 12 weeks. Unfortunately, some very rare individuals may shed virus for longer periods of time.<sup>6,44</sup> This author has identified a pair of Nanday Conures and two Bourke's Parakeets that were found shedding virus on three cloacal samples six months apart.<sup>44</sup> If long-term virus shedding is an actual phenomenon, even in an extremely small percentage of infected birds, testing of all birds or careful nursery management will be essential in preventing nestling exposure.

Another management tool that may prevent APV disease in the nursery would be to pull all eggs from the adults and incubator hatch them. As has been discussed before, this author feels that egg transmission is either rare or nonexistent.

## Preventing APV Infection and Disease in Budgerigar Aviaries

1. Make sure that APV is not

already present. Select a representative number of birds in the collection and have them tested by serology for evidence of infection.

2. All budgerigars entering the aviary should be seronegative.

3. Carefully restrict all movement of birds on and off the property.

a. If the aviary is a commercial aviary, dealers, feed sales persons, delivery trucks, and other bird breeders should be banned from the aviary entirely. Young birds taken to the bird dealer and rejected should not be returned to the aviary.

b. If the aviary is primarily breeding show Budgerigars, then all birds going to the show should be quarantined until the end of the show season and tested by serology before they are returned to the breeding colony.

c. A modified-live vaccine may be available sometime in the future for budgerigars.<sup>55</sup> This vaccine may prove useful for show Budgerigars. Show birds would need to be immunized at least a month before the show season was to begin. Until the value of this vaccine is proved, these birds should be tested by cloacal swab or blood PCR before returned to the collection.

d. The potential use of a modified live vaccine in a commercial flock has been suggested, but its actual value will need to be proved. Immunizing thousands of birds will be labor intensive and potentially very expensive. Again, it will only benefit aviaries that are initially free of the disease and not infected birds.

## Preventing APV Disease in the Pet Store

The pet store is one of the most common places where APV outbreaks occur. Most pet stores get their birds from multiple sources, they sell Budgerigars, lovebirds, and Cockatiels, the three species that are most likely to be shedding virus, and many stores will acquire susceptible species when they are still nestlings. To avoid disease, pet stores can use several strategies.

1. The easiest and best method for preventing APV disease in the pet store is to buy only weaned nestlings.

These birds will be old enough that if infected with APV they will not develop disease.

2. If unweaned nestlings are to be purchased, they should be raised outside of the store until weaned.

3. If nestlings must be in the store, they should be separated from all other birds, and a person designated to take care of them and no other birds. The public should not be allowed to handle these birds.

4. Early immunization of nestlings (at five and seven weeks) did not induce an antibody titer in birds immunized by this author. Therefore, we should be very cautious and not assume nestlings vaccinated at this young age are protected from APV infection.

### Control of APV Outbreaks

#### Control in the Nonbudgerigar Aviary

In most cases, once APV is introduced to a nursery it spreads rapidly, so that by the time the first case is recognized most of the nestlings are already infected. This concept is important for two reasons. First, vaccination at this point will do no good. Second, testing during the outbreak will only prove that the virus is widely disseminated. To save money, in most cases, the aviculturist should be encouraged to reserve testing to determine when shedding is stopped and the chicks can be sold.

Little can be done to keep exposed chicks from disease. However, efforts should be made to improve hygiene, spread out birds, use individual syringes for handfeeding individual chicks. The most important element in the control of APV outbreaks is to stop bringing babies into the nursery. Chicks can be left in the nest to be raised by the parents or pulled and sent to another facility to be raised. It remains unclear why, but parent-raised chicks (excepting lovebirds and Budgerigars) are not reported to develop APV disease. Surviving chicks will shed virus for eight to 14 weeks, rarely as long as 16 weeks. All chicks should be found negative by blood PCR and then held for an additional two weeks before being sold.

After the outbreak has stopped, a

close inspection of the aviary must be done. Possible sources of the virus need to be identified and tested or eliminated from the aviary. Extensive cleaning and disinfection of the nursery will also have to be done. In aviaries where the underlying source of disease has been eliminated, subsequent breeding seasons can be free of the disease.

#### Control of APV in Budgerigar Aviaries

The cycle of infection and disease in the Budgerigar aviary is maintained by virus shedding of young adult birds and nestlings.<sup>35</sup> The shed virus contaminates the environment and young birds are probably infected as soon as they hatch. To break the cycle, breeding should be stopped, the young birds removed from the aviary, and the experienced adult birds moved to a clean environment. After several months, if the facility is adequately disinfected, the established breeders can be put to work again.<sup>42</sup>

It is important to note that disinfecting a small barn, shed, or other wooden structure and wooden nest boxes is difficult at best. The use of formaldehyde gas may be necessary. This type of disinfection must only be done by someone with extensive experience with this highly toxic agent.

#### APV Infection and Disease in Non-psittacine Birds

There is no doubt that one or more avian polyomaviruses can infect non-

psittacine birds. Several species of passerines have been documented to have classical APV disease.<sup>11, 12, 13, 19, 21, 27, 63</sup>

In the author's experience, flocks of Gouldian Finches are perhaps at greatest risk. Again in the author's experience, mortality is limited to nestling and young adult finches during one breeding season, but is not seen again in the following year. Surviving birds have moderate levels of antibody that will neutralize a lovebird derived APV. APV DNA was detected in the tissues of one finch with PCR primers derived from the psittacine APV sequence, suggesting that this bird was infected with a psittacine variant. However, other studies suggest that another significantly different virus may also infect passerines.

Recently, a Green Aracari has been documented with APV disease. Sequence analysis of this virus suggests that it was a psittacine APV that for some unknown reason crossed over into an aberrant host. As the bird's mate never developed evidence of infection, it was postulated that the infected bird may have been immunosuppressed.<sup>23</sup>

It is extremely disturbing, that APV has recently been documented in chickens in Europe<sup>60</sup> and the United States.<sup>16</sup> How this virus has reached these populations is not known. This author, however, was provided with sections of a house sparrow *Passer domesticus* from Maryland.<sup>41</sup> This bird had characteristic lesions of APV disease, raising the possibility of APV infection in wild birds.

Table 2

### Risk Factors Associated With APV Outbreaks

1. Exposure at bird shows, sales, and fairs.
2. Movement of birds in and out of the aviary.
3. Mixed collections of birds. Especially those containing lovebirds, budgerigars, and cockatiels.
4. Psittacine Beak and Feather Virus infected birds on the premises.
5. Chicks from various sources being raised in the same nursery.
6. Birds of susceptible ages in pet stores.
7. Failure to quarantine new birds or inappropriate quarantine procedures.
8. Failure to test new birds brought into the aviary.

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## Conclusions

The avian polyomavirus is a single virus with a broad host range. Its ability to infect and cause disease in birds is dependent on the age of the bird, the species of the bird, the immune status of the bird, and other poorly understood factors. It is first necessary to understand the complex biology of this virus before the practitioner or the aviculturist can begin to choose the appropriate strategies to control it.

It is hoped that this article will result in an open and frank discourse about what we know and do not know about the control of APV. None of us knows all there is to know about APV and new findings will undoubtedly modify our understanding of it. It is therefore essential that all views in the discussion of this virus and disease be heard and that all possibilities be considered.

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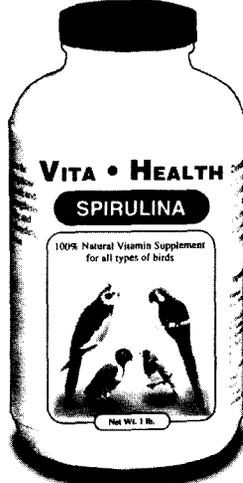
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