

Viremia, virus shedding, and antibody response during natural avian polyomavirus infection in parrots

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Objective—To determine rapidity of spread and onset and duration of viremia, virus shedding, and antibody production in parrots naturally infected with avian polyomavirus (APV).

Design—Case series.

Animals—92 parrots in 2 aviaries.

Procedure—Blood samples were obtained from parrots naturally exposed to APV during a 3- to 4-month period for determination of serum virus neutralizing antibody and detection of viral DNA. Nestlings from the next year's hatch were monitored for APV infection.

Results—The first indication of inapparent infection was viremia, which developed simultaneously with or was followed within 1 week by cloacal virus shedding and antibody production. Cloacal virus shedding continued after viremia ceased. During viremia, viral DNA was detected continuously in blood samples. Viral DNA was detected in serial cloacal swab specimens in most birds, but it was detected inconsistently in 6 birds and not detected in 3 birds, even though these birds were viremic. Duration of cloacal virus shedding was ≤ 4.5 months. In 1 aviary, prevalence of infection was 88% and dissemination of virus through the 3-room building required 4.5 months. In the second aviary, a single-room nursery, prevalence of infection was $\geq 90\%$. For all affected birds, infection could be detected 18 days after the first death.

Conclusions and Clinical Relevance—If a single sampling is used for polymerase chain reaction detection of viral DNA, blood and cloacal swab specimens are required. In nestling nonbudgerigar parrots, cloacal virus shedding may persist for 4.5 months. Management protocols alone are sufficient to prevent introduction of APV into a nursery. (*J Am Vet Med Assoc* 2000;217:32–36)

Avian polyomavirus (APV) is a highly infectious virus of psittacine birds. In a nursery or indoor aviary the virus spreads widely, resulting in infection rates that commonly approach 100%.^{1,2} Whether infection results in disease is dependent on age, species, and individual characteristics of the infected bird.³⁻⁵ In most outbreaks, disease is confined to Macaws (*Anodor-*

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Supported by the Department of Large Animal Medicine and Surgery and the Schubot Exotic Bird Health Center, Texas A&M University, and Research Associates Laboratory, Milford, Ohio.

hynchus hyacinthinus and *Ara* spp), Conures (*Aratinga* spp, *Nendayus nenday*, and *Pyrrhura* spp), Eclectus Parrots (*Eclectus roratus*), Ring-neck Parakeets (*Psittacula krameri*),^{5,8} Lovebirds (*Agapornis* sp),^{8,9} and Budgerigars (*Melopsittacus undulatus*).^{10,11} Affected Budgerigars typically die between 10 and 20 days of age,^{10,11} affected Conures die between 1 and 6 weeks⁵⁻⁸ of age, and affected Macaws and Eclectus Parrots die between 4 and 14 weeks of age.^{5,6,8} Disease in adult birds is rare¹²⁻¹⁴ and is often associated with a concurrent infection with psittacine beak and feather disease virus (PBFDV), which is thought to be immunosuppressive.^{13,14} For unknown reasons, a certain percentage of APV infections in individuals of susceptible species and susceptible age are inapparent.^{1,7,8}

Nestlings and adult birds with inapparent infections have viral DNA in the cloaca and are assumed to be shedding virus.¹⁴ These birds are likely sources of outbreaks that occur after bird shows and sales, introduction of nestlings from multiple sources into a nursery, or introduction of susceptible nestlings into pet stores. Detection of infected birds that shed or will shed virus is a key element in the prevention of dissemination of APV.

Inapparently infected birds have been detected by use of polymerase chain reaction (PCR) assays of cloacal swabs (cloaca PCR)^{4,7} and a PCR assay of blood (blood PCR).⁷ To date, there have only been limited studies that examine the duration of viremia and virus shedding and compare the abilities of these assays to detect inapparently infected birds.^{1,2,4,7,15} The data obtained from these studies suggests that the duration of viremia and virus shedding is dependent on the individual bird infected, the species of bird infected, and, possibly, age at time of infection.

The purpose of the study reported here was to determine rapidity of spread, and onset and duration of viremia, virus shedding, and antibody production in parrots naturally infected with APV.

Materials and Methods

Aviaries—Aviary 1 included breeding pairs and replacement stock that were housed outdoors in individual cages, and a nursery (3 bird rooms, connected by a kitchen) that was located away from the breeding birds. Only birds in the nursery were studied. Infection with APV had never been detected in the aviary. At the time of the outbreak, 8 nestling Blue and Gold Macaws (*Ara ararauna*; ages, 6 to 8 weeks) were housed in the central room of the nursery, 2 Red-Fronted Macaws (*Ara rubrogenys*; age, 12 weeks) were housed in an adjacent room, and in the last room an Umbrella Cockatoo (*Cacatua alba*), 3 Hyacinth Macaws (*A hyacinthinus*), 2 Blue and Gold Macaws, and a Red-Fronted

Macaw were housed. These 7 birds ranged in age from 18 months to 7 years.

Three weeks before the first death in aviary 1, 17 nestlings were transferred off the property to a second nursery where they were held for 8 days. Two weeks before the first death, 4 Blue and Gold Macaw nestlings were taken to a bird mart where they were in close proximity to many other birds of various ages. All birds were returned to the aviary 12 days before the first death. Two days before the first death, 8 older Macaws (12 to 14 weeks old) were shipped to a pet store in another state. These birds remained healthy but were never tested for APV infection.

Aviary 2 included a mixed collection of > 200 pairs of birds (14 species), most of which were parrots. Breeding pairs were housed in outdoor flight cages and several small indoor enclosures. Until 3 weeks prior to the APV outbreak, all eggs were incubator hatched, and nestlings were raised in a 1-room nursery that was isolated from the breeding stock. Three weeks before the first death, 2 parent-raised African Grey Parrots from another aviary were introduced into the nursery. At the time of the first death, the nursery contained 38 nestling parrots, including 4 Blue-Crowned Conures (*Aratinga acuticaudata*), 2 Blue and Gold Macaws, 2 Amazon Parrots (*Amazona* spp) 1 African Grey Parrot (*Psittacus erithacus*), 2 Eclectus Parrots, and 27 Cockatoos (*Cacatua* spp) of 7 species. Age of the nestlings ranged from 13 to 72 days (median age, 40 days).

Sample collection and analysis—At each sampling, the cloacal mucosa of each bird was swabbed with a sterile swab. One milliliter of blood was collected from the right jugular vein, and equal aliquots were placed in 2 containers with heparin as an anticoagulant. Swab specimens and blood were shipped overnight on ice for detection of APV DNA by use of a proprietary PCR test⁶ that is sufficiently sensitive to detect between 4 and 10 copies of APV genome.⁷

Plasma from the second aliquot of blood was used in a virus neutralization assay.² Virus neutralization titers were expressed as the reciprocal of the serum dilution resulting in a 50% reduction in cytopathic effect.

Samples were collected from each bird in aviary 1 for blood and cloacal PCR and serologic testing 2, 11, 27, 43, 103, and 127 days after the death of the index case. The number of birds from which samples were obtained decreased from 15 on day 2 to 11 on day 103 because 4 additional Blue and Gold Macaw chicks died during this time. Samples were not obtained from a single Hyacinth Macaw on day 43 because of hematoma formation at the venipuncture site. In aviary 2, samples were obtained from 30 birds for blood and cloacal PCR and serologic testing 1 to 4 times 12, 18, 35, and 76 days after the death of the index case.

Confirmation of APV-induced disease—Avian polyomavirus-induced disease was confirmed in birds that died by detection of gross lesions of disseminated hemorrhage, characteristic microscopic lesions in liver, spleen, and kidney, and high concentrations of APV DNA detected in liver tissue by use of PCR.^{6,15,16}

Serum biochemical analysis and thrombocyte evaluation—Blood was collected from 2 Blue and Gold Macaw nestlings in aviary 1 within the hour prior to death. Activities of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), γ -glutamyl transferase (GGT), and creatinine phosphokinase (CPK) were determined for each sample. Activity of lactose dehydrogenase (LD) was only evaluated in 1 bird. A blood smear was made from fresh blood that did not contain anticoagulants, for each bird.

Follow-up samples—In the year following each outbreak, blood was obtained from 9-week-old nestlings from

each aviary for serologic and PCR tests. Cloacal swab specimens were not obtained. In aviary 1, samples were obtained from 20 Macaws of 4 *Ara* species. In aviary 2, samples were obtained from 1 Black-Headed Caique (*Pionites melanocephala*), 4 Amazon Parrots (*Amazona* spp), 2 White-Eared Conures (*Pyrrhura leucotis*), 2 Blue and Gold Macaws, 2 Chattering Lories (*Lorius garrulus*), and 5 Cockatoos (*Cacatua* spp).

Statistical analyses—For birds with patent infections (ie, those with positive results of PCR tests of blood specimens, cloacal specimens, or both), the number of blood samples with positive results of PCR for viral DNA was compared with the number of cloacal swab specimens with positive results of PCR, by use of the χ^2 test of independence.¹⁷ Differences were considered significant at $P < 0.05$.

Results

Aviary 1—The index case was represented by a nestling Blue and Gold Macaw that died 14 days after the bird was taken temporarily to a bird mart; 4 other Blue and Gold Macaws also died (days 12, 23, 33, and 55). Results of PCR assays and serologic testing were depicted graphically (Fig 1). Maximal antibody titers ranged from 1:512 (an Umbrella Cockatoo) to 1:8,192 (a nestling Blue and Gold Macaw). Median maximal antibody titer was 1:4,096. New nestlings were not placed in the nursery during the outbreak, and the

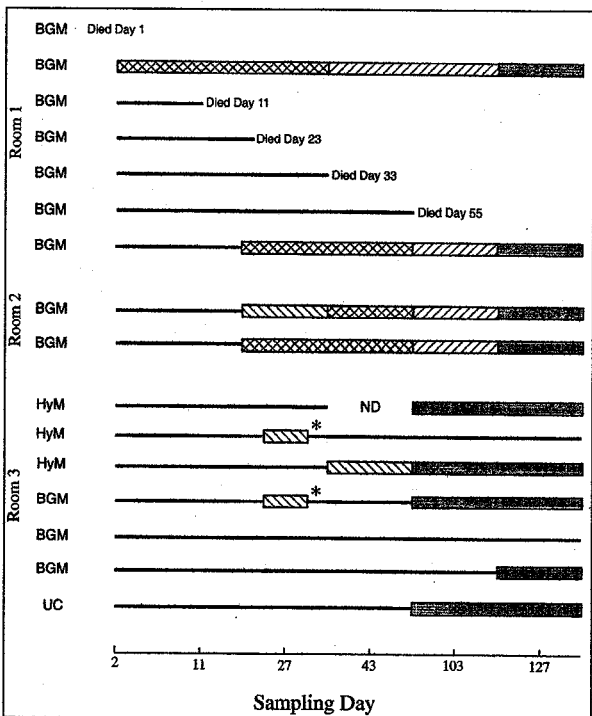


Figure 1—Results of serologic tests and polymerase chain reaction (PCR) tests of blood and cloacal swab specimens for avian polyomavirus infection in parrots in 3 rooms of an aviary (aviary 1). Parrots were 5 to 8 weeks old (room 1), 14 weeks old (room 2), or > 1 year old (room 3). Results were negative for all tests (—), positive for blood by PCR testing (▨), positive for cloacal swab specimens by PCR testing (▨), or positive for blood and cloacal specimens by PCR testing (▨). Unshaded bars indicate negative serologic results; shaded bars indicate positive serologic results. BGM = Blue and Gold Macaw. HyM = Hyacinth Macaw. UC = Umbrella Cockatoo. ND = Not done. * = False-positive results caused by sample contamination.

nursery was vacated and disinfected after the birds ceased shedding virus. In the subsequent breeding season, nestlings left the aviary only after being sold and were not allowed to re-enter the nursery. Blood samples were obtained from 17 nestlings in the subsequent breeding season; results of serologic tests and PCR were negative. There was no mortality in the nursery in the following year.

Serum biochemical values for the 2 Blue and Gold Macaws from which samples were obtained immediately prior to death were: AST, 3,106 and 2,865 U/L (reference range, 53 to 147 U/L)¹⁸; ALT, 557 and 305 (reference limit, < 10 U/L)¹⁸; AP, 684 and 816 U/L (reference range, 400 to 2000 U/L)¹⁸; LDH, 4,412 U/L (second Macaw only; reference limit, < 340 U/L)¹⁸; GGT, 9 and 8 U/L (reference limit, < 10 U/L)¹⁹; CPK, 576 and 396 U/L (reference limit, < 1,100 U/L).¹⁸ A blood smear from each bird was examined in its entirety; thrombocytes were not detected.

Aviary 2—The first bird that died (day 0) was a Blue and Gold Macaw (age, 5 weeks). An Eclectus Parrot (age, 7 weeks) and a Blue and Gold Macaw (age, 5 weeks) died on day 1 and 3, a Blue-Crowned Conure (age, 4 weeks) died on day 4. In addition, two 17-day-old Umbrella Cockatoos developed crop stasis and became dehydrated on day 4; these birds responded clinically after administration of fluid therapy for 3 days. Both birds developed a generalized feather dystrophy during the next 4 weeks. Results of serologic tests and PCR on blood and cloacal swab specimens indicated that both birds were infected with APV, whereas negative PCR results for PBFDV^a were obtained from blood samples. Two birds generally considered to be of susceptible age and species,^{3,5} a Blue and Gold Macaw and an Eclectus Parrot, survived without developing clinical signs. Results of PCR and virus neutralization assays on the surviving birds from aviary 2 were determined (Fig 2). Samples were not obtained from all birds at each sampling, and some birds were taken off the premises during the study. At the end of the breeding season, the nursery was disin-

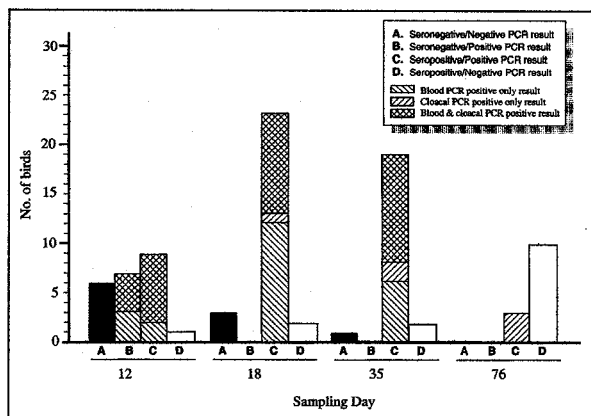


Figure 2—Results of virus serum neutralization tests and detection of viral DNA by use of PCR testing in psittacine birds naturally infected with avian polyomavirus (aviary 2). Blood and cloacal swab specimens were collected on various days after detection of the first death in an aviary. Seropositive = 50% reduction of cytopathic effect at serum dilution of 1:16.

fecting; in the following breeding season, only incubator-hatched chicks were raised in the nursery. Blood samples obtained from 16 nestlings the year after the outbreak yielded negative results for serologic tests and PCR.

Combined data—All birds that died in each aviary were necropsied and their tissues examined histologically and by PCR. Each bird had characteristic gross lesions consisting of widespread hemorrhage. Microscopically, APV infection was confirmed by the characteristic pattern of hepatic necrosis and APV-specific inclusion bodies in the spleen and, in some instances, the mesangial cells of the kidney. Polymerase chain reaction of liver and spleen specimens revealed high concentrations of APV DNA (> 1 × 10⁶ copies of virus/μg of host DNA).

With the exception of 2 birds from aviary 1, all birds with positive results for PCR also seroconverted; the 2 birds (a Hyacinth Macaw and a Blue and Gold Macaw) that did not seroconvert had positive results for PCR only once and only with a blood sample. Five birds seroconverted but never had positive results for PCR. If seroconversion was considered indicative of infection, prevalence of infection in aviary 1 was 86%. The exact prevalence of infection in aviary 2 could not be calculated because 3 birds with negative results for PCR and serologic tests were removed from the nursery between the third and fourth sampling. All of the remaining 28 birds from which samples were obtained seroconverted; therefore, minimum prevalence of infection in aviary 2 was 90%.

Combining the data from both aviaries, the first indication of infection in 12 birds was detection of APV DNA in blood by use of PCR. For 18 birds, initial results were positive for PCR performed on blood and cloacal swab specimens. Five birds seroconverted but never had positive results for PCR.

If each sampling (blood and cloacal PCR) from each bird was taken as a separate and independent event, there were 73 samplings for which positive results were obtained by 1 or both of these assays. Of these 73 samplings, 25 (34%) samples yielded positive results by PCR analysis of blood only, 10 (14%) yielded positive results by PCR analysis of cloacal swab specimens only, and 38 (52%) yielded positive results with both assays. Had PCR analysis at each of the 73 samplings been limited to a blood sample or a cloacal swab specimen, but not both, 63 (86%) blood samples and 48 (65%) cloacal swab specimens would have yielded positive results. Results of the χ^2 test of independence indicated that the number of positive PCR results for blood samples was significantly greater than that of cloacal swab specimens. Six birds had consistently positive results for blood samples on 3 consecutive samplings but had positive results for cloacal swab specimens only at the first and third samplings. Three birds had positive PCR results for blood samples on 3 samplings but never had positive results for cloacal swab specimens.

Discussion

To develop proper strategies for detecting parrots with inapparent APV infection, it is necessary to know the onset, duration, and frequency of virus shedding in

naturally infected birds. Previous data suggest that these variables may differ between species of birds and birds of different ages. In the study reported here, we were able to follow the onset and duration of viremia, virus shedding, and humoral immunity in 39 parrots (14 species) naturally infected with APV.

In aviary 1, the first fatally infected bird died 14 days after the suspected date of exposure; 2 days later an inapparently infected Macaw was viremic and shedding virus. These findings are consistent with other reports suggesting that the period between infection and the onset of disease in nonbudgerigar parrots naturally infected with APV is approximately 10 to 14 days.⁸

In Budgerigars, the period between infection and disease or the onset of virus shedding is approximately 7 days.⁸ The duration of this period in naturally infected nonbudgerigar parrots is unknown. When parrots are experimentally infected by IM or IV administration of APV, virus can be detected in specimens obtained from the cloaca in as little as 2 days¹⁸; it is not known how closely these experiments approximate the conditions of natural infection. Because the period between the first death and subsequent deaths and detection of inapparent infections was ≥ 11 days, we believe that only 2 Blue and Gold Macaws were exposed at the bird show, and that they were the source of infection for other birds in the nursery.

The source and date of first exposure in aviary 2 could not be determined. It is possible that 2 African Grey Parrots that were introduced into the nursery 3 weeks prior to the APV outbreak were the source of infection; they were not tested, however, so proof was lacking.

The data in this report suggest that viremia precedes cloacal virus shedding, because viremia was always detected prior to or concurrent with cloacal virus shedding. The duration between onset of viremia and onset of cloacal virus shedding appears to be only a few days in a typical infection, because most birds that were viremic and not shedding virus initially were viremic and shedding virus at the second sampling. Two birds were viremic on 3 consecutive samplings, but virus was never detected in cloacal swab specimens, suggesting that some birds may be infected but may not shed virus. Viremia was consistently detected in all birds until the viremia was cleared; after that time, virus was not detected in the blood again. Cloacal virus shedding was intermittent in some birds, but often persisted after cessation of viremia.

The duration of viremia and cloacal virus shedding could not be calculated specifically in this study because of the prolonged intervals between some samplings, because some birds were viremic and shedding prior to the first sample, and because some birds were still shedding at the last sampling. Nevertheless, some general observations were possible. In nestlings, duration of viremia was always > 17 days and < 64 days. Duration of cloacal virus shedding in nestlings was > 17 days but < 76 days; most nestlings shed virus for < 64 days. An exception was a Blue and Gold Macaw that shed virus for 108 to 135 days.

Only 7 adult birds were studied; of these, 2 did not seroconvert, viremia was detected in only 1, and virus

shedding was detected in none. The adult birds only became infected late in the study, and samples were obtained less frequently than for the juvenile birds. Therefore short periods of virus shedding (< 60 days) may have occurred, but were missed.

Combined data from the 2 aviaries suggested that neither PCR test alone (ie, using blood or cloacal swab specimens) will detect all birds actively infected with APV. Neither test would detect infected birds during the prepatent period (ie, period between exposure and viremia). After the prepatent period, results of PCR with blood samples became positive first and remained consistently positive until viremia was cleared; results of PCR with cloacal swab specimens became positive shortly thereafter in most birds, but were inconsistently positive in some birds.

Because serial testing is not an economical or practical option for most bird owners, birds potentially exposed to APV should not be tested until at least 2 weeks after exposure to avoid missing prepatent infections. To detect all patently infected birds, blood and cloacal swab specimens should be tested. If quarantine is substituted for testing, results of the study reported here suggest that the quarantine period should be at least 135 days. Results of another study suggest that the quarantine period should be much longer (≥ 6 months) in Budgerigars.¹ Healthy Cockatiels and a few individuals of other parrot species may shed virus intermittently or continuously for 18 months or more, and testing is the only practical way to prevent such birds from introducing APV into a collection.^{1,7} It has been postulated that birds with concurrent APV and PBFDV infections may shed virus continuously over long periods of time.⁸ True or not, birds at high risk for PBFDV infection should be screened for PBFDV prior to introduction to an aviary.

In both aviaries, viremia persisted despite high virus neutralizing antibody titers, suggesting that cell-mediated rather than humoral immunity may be necessary to clear infection, and that vaccination of infected birds with commercial inactivated vaccine would not alter the course of the infection.

In aviary 1, two Blue and Gold Macaws became infected and did not develop clinical disease, although they were the same age as other Blue and Gold Macaws that died. Similarly, older Red-Fronted Macaws became infected but did not develop disease. In aviary 2, although Eclectus, Macaw, and Conure chicks developed disease and died, only 2 young Cockatoos had transient clinical signs; the Amazon Parrots and an African Grey Parrot were also inapparently infected. These results are consistent with earlier observations that some infected birds do not develop clinical disease, and that development of disease is dependent on the species of birds and their age, as well as undefined individual factors.^{1,4,7,8}

In a recent study, the use of a commercial avian polyomavirus vaccine was suggested to be instrumental in ending outbreaks of APV disease in 9 parrot nurseries.²⁰ The authors reported that in each aviary mortality ceased within 2 weeks of the second vaccination (4 weeks after the first vaccination). In addition to vaccination, the authors made substantial management

changes in each aviary. Control flocks in which management changes alone were made or in which a placebo was used were not included in the study. Although it was implied that the vaccine prevented infection in these birds, none of the vaccinated birds were tested by PCR analysis of blood or cloacal swab specimens to verify this assumption. Results of the study reported here, however, in which new infections were apparently prevented by avoiding introduction of new nestlings into the aviaries, suggest that the effects attributed to vaccination may have been the result of rapid dissemination of virus within the flock, resulting in the deaths of susceptible birds and inapparent infection of the remaining birds.

The substantial increase in serum activities of AST and LDH, without increased CPK activity, is consistent with the observed hepatic necrosis.^{19,21-23} The observed increase in serum activity of ALT remains unexplained, because it is not thought to be present in substantial concentrations in the liver.^{19,22-24}

Terminal widespread bleeding is characteristic of disease caused by APV,^{3,5,6} although cause of this apparent clotting disorder is not known. For 2 birds from which antemortem blood samples were collected, thrombocytes were not detected in blood smears made directly from the needle with which the blood was collected. Actual thrombocyte counts were not performed because conventional methods used for evaluating mammalian platelets cannot be used in birds.¹⁹ The apparent thrombocytopenia detected in the study reported here may explain why birds with APV-induced disease bleed. Cause of the thrombocytopenia, however, remains unknown; it may result from viral destruction of thrombocytes or their precursors, or represent the result of virally induced disseminated intravascular coagulation.

Two unexplained positive results of PCR analysis were detected in aviary 1. One Hyacinth Macaw had positive results for PCR with a blood sample but did not seroconvert. One Blue and Gold Macaw had positive results for PCR only once, > 17 days before it seroconverted. The most likely explanation for these positive results is that they represent environmental contamination at the time of collection. Because of the sensitivity of the PCR, contamination is a constant concern when samples are obtained from multiple birds.

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