A Survey of Avian Polyomavirus (APV) Infection in Imported and Domestic Bred Psittacine Birds in Japan

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ABSTRACT. Although birds infected with avian polyomavirus (APV) subclinically could be a source of infection, no epidemiological studies of APV in psittacine birds have been reported in Japan. In the present study, we investigated subclinical morbidity rate of APV in imported and domestically bred psittacine birds by polymerase chain reaction (PCR). Of 402 live birds from which blood or feather samples were taken between April, 2003 and March, 2004, 11 (2.7%) were found to be APV positive. The DNA sequences of the APV t/T antigen region were determined for five APV-positive randomly selected samples and were found to be conserved.

NOTE. Avian polyomavirus (APV) is known as the causative agent of budgerigar fledgling disease (BFD) which is characterized by feather abnormalities and loss of fledgling and young budgerigars [5]. This disease has been observed worldwide since its first recognition in the early 1980s [4, 5, 8]. The host range of APV is wide and many birds are susceptible including not only budgerigars but also non-budgerigar psittaciformes and other many avian orders [6, 7, 12, 16]. APV infections in non-budgerigar psittacine birds may cause peracute death with subclinical signs and less feather abnormalities [11]. A commercial inactivated vaccine for APV infection is available in the U.S.A.

Psittacine birds are among the most popular companion animals in Japan. Psittacine species are imported from many countries and some are bred in domestic farms. Imported psittacine birds appear to be healthy. Presently, APV infection cannot be found without feather abnormalities. Birds infected with APV subclinically could be a source of infection. A worldwide epidemiological study is needed, however no epidemiological studies have been reported in Japan. In the present study, we investigated the subclinical morbidity rate of APV in imported and domestically bred psittacine birds by polymerase chain reaction (PCR).

We examined 402 live psittacine birds between April, 2003 and March, 2004. These birds included 247 from Republic of South Africa, 48 from Singapore, 36 from Indonesia, 4 from European countries, 3 from the U.S.A., 2 from Guyana, 20 from unknown countries, and 42 that were bred in Japan. None of the birds had feather disorders. The birds examined included 107 males, 99 females and 196 birds of unknown sex. The birds included 72 birds under 3 months old (nestlings), 206 birds between 3 months and one year old (young), 32 birds over one-year-old (young adults) and 92 birds of unknown age. Species of birds were classified according to Rowley [13] and Collar [3].

For DNA sources, we used blood or feathers, as was done in epidemiological studies in Italy [2] and Germany [10]. Blood samples were taken from 348 birds and feather samples were taken from 54 birds. For the imported birds, blood and feather samples were taken within 4 days after importation.

DNA was extracted from blood and feathers with a SepaGene nucleic acid extraction kit (Sanko Junyaku Co., Tokyo, Japan) according to the manufacturer’s instructions. Feathers were first pretreated for 3 hr at 55°C in solution I (Tris-HCl, pH 7.95) with 1% Sodium Lauryl Sulfate and 100 µg/ml of Proteinase K. DNA extracted from the blood and feathers was dissolved in 30 µl of 10 mM Tris-1 mM EDTA (pH 8.0) and stored at –30°C until use. A 310-base pair (bp) fragment corresponding to the t antigen encoding and T antigen gene intron region of the APV was amplified by PCR using primers Forward (5’-CAA GCA TAT GTC CCT TTA TTC CCA GAT G-3’) and Reverse (5’-CTG TTT AAG GCC TTC CCA GAT G-3’) [6]. In order to prepare templates for sequence analysis, DNA samples of five randomly chosen APV-positive samples were amplified with another pair of primers, BFDDupF (5’-CAG GCC TTA TAT CCT GTT TGC GTC-3’) and BFDDupR (5’-GAT ATC AAG ACT GCC TAT CGT CGC-3’) which were designed to amplify a 298-bp fragment corresponding to the 5’ part of the t and T antigen-encoding region [9]. PCR products were cloned and sequenced as described previously [9]. Accession numbers of sequences presented in this paper are AB241069 to AB241073. Seven APV published sequences (Accession Nos. AB182561 to AB182567) were used for comparison.

The 402 birds represented 20 genera from two families (Table 1). A total of 11 APV-positive birds were found in...
six genera (Table 1). The 11 APV-positive birds were of various species from South Africa, Japan, U.S.A. and unknown countries (Table 2). The rate of APV infection (2.7%) that we examined was higher than the rates observed in studies of psittacine birds in Italy (0.8% in 877 birds) [2] and Germany (0% in 85 birds) [10]. No comparable data has previously been collected in Japan. Four birds imported from South Africa were APV-positive. Albertyn et al. [1] were unable to find any reports of APV infection in South Africa at the time of their study, this may be the first detection of APV subclinical infection of psittacine birds in South Africa. However, we cannot rule out the possibility that the infected South African birds examined in this study were infected by birds from different origins during importation.

The APV T antigen region was expected to be conserved based on our previous study [9]. Five of the present APV-positive samples were randomly selected and sequenced to test this assumption (Table 3). Four of the present sequences (RRP-JP2A, BP-SA1A, BYM-US1A and RFP-SA1A) were the same each other and they were also
the same as five of our previous sequences (OL-SI1A, EP-JP1A, RRP-JP11A, RRP-JP12A and BTP-SA11A). Two of the remaining sequences (GCP-JP2A and GCP-JP1A, both from Green-cheeked parakeets in Japan) had a C-to-T transition at nucleotide 2. One sequence (GFM-1) had a G-to-T transversion at position 140. None of these substitutions changed the predicted amino acid sequence. Our data are insufficient to tell whether the substitution at nt 2 in Green-cheeked parakeets in Japan is related to the host range.

Our sequencing results are consistent with the finding that the APV genome is highly conserved among strains [14, 15]. This lack of variation in the APV sequences may make it difficult to determine the source of APV with a molecular epidemiological approach. In conclusion, we detected 11 APV-positive birds out of 402 birds examined. This study represents a wide-area survey of APV infection because most of the psittacine birds examined in this study were imported to Japan from other countries.

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REFERENCES


**Table 3. APV used for sequence analysis and the positions of nucleotide (nt) substitutions**

<table>
<thead>
<tr>
<th>Code</th>
<th>Host</th>
<th>nt 2</th>
<th>nt 140</th>
<th>Origin</th>
<th>Accession no.</th>
<th>References</th>
</tr>
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<tr>
<td>GCP-JP2A</td>
<td>Green-cheeked Parakeet (<em>Pyrrhura molinae</em>)</td>
<td>T</td>
<td>G</td>
<td>Japan a)</td>
<td>AB241069</td>
<td>This study</td>
</tr>
<tr>
<td>RRP-JP2A</td>
<td>Rose-ringed Parakeet (<em>Psittacula krameri</em>)</td>
<td>C</td>
<td>G</td>
<td>Hyogo, Japan</td>
<td>AB241070</td>
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</tr>
<tr>
<td>BP-SA1A</td>
<td>Brown Parrot (<em>Psitacula gualiemi</em>)</td>
<td>C</td>
<td>G</td>
<td>South Africa</td>
<td>AB241071</td>
<td>This study</td>
</tr>
<tr>
<td>BYM-US1A</td>
<td>Blue-and-yellow Macaw (<em>Ara ararauna</em>)</td>
<td>C</td>
<td>G</td>
<td>U.S.A.</td>
<td>AB241072</td>
<td>This study</td>
</tr>
<tr>
<td>RFP-SA1A</td>
<td>Red-fronted Parrot (<em>Psitacula gualiemi</em>)</td>
<td>C</td>
<td>G</td>
<td>South Africa</td>
<td>AB241073</td>
<td>This study</td>
</tr>
<tr>
<td>OL-SI1A</td>
<td>Ornate Lorikeet (<em>Trichoglossus ornatus</em>)</td>
<td>C</td>
<td>G</td>
<td>Singapore</td>
<td>AB182561</td>
<td>Ogawa et al. [9]</td>
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<tr>
<td>GCP-JP1A</td>
<td>Green-cheeked Parakeet (<em>Pyrrhura molinae</em>)</td>
<td>T</td>
<td>G</td>
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<td>C</td>
<td>G</td>
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<td>Ogawa et al. [9]</td>
</tr>
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<td>BTP-SA11A</td>
<td>Brown-throated Parakeet (<em>Aratinga pertinax</em>)</td>
<td>C</td>
<td>G</td>
<td>South Africa</td>
<td>AB182566</td>
<td>Ogawa et al. [9]</td>
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<tr>
<td>GFM-1</td>
<td>Budgerigar (<em>Melopsittacus undulatus</em>)</td>
<td>C</td>
<td>T</td>
<td>Shizuoka, Japan</td>
<td>AB182567</td>
<td>Hirai et al. [5]</td>
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a) Prefecture unknown.