



Avian Polyomavirus Genome Sequences Recovered from Parrots in Captive Breeding Facilities in Poland

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Eight genomes of avian polyomaviruses (APVs) were recovered and sequenced from deceased *Psittacula eupatria*, *Psittacula krameri*, and *Melopsittacus undulatus* from various breeding facilities in Poland. Of these APV-positive samples, six had previously tested positive for beak and feather disease virus (BFDV) and/or parrot hepatitis B virus (PHBV).

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olyomaviruses (family *Polyomaviridae*) are nonenveloped viruses with an icosahedral capsid of ~45 nm in diameter and a circular double-stranded DNA genome of ~5 kb. The bidirectionally transcribed circular genome encodes three structural proteins, VP1, VP2, and VP3, on one strand, and transforming nonstructural protein genes, the large and small T antigens, on the complementary strand. Numerous polyomaviruses have been identified and infect a wide range of vertebrates. Currently, all known polyomaviruses can be assigned to three genera: Orthopolyomavirus and Wukipolyomavirus, which encompass polyomaviruses of mammalian origin, and Avipolyomavirus, which infects birds. Documented avipolomaviruses include Adélie penguin polyomavirus, butcherbird polyomavirus, canary polyomavirus, crow polyomavirus, finch polyomavirus, goose hemorrhagic polyomavirus, and the avian polyomaviruses (APVs) (formerly known as budgerigar fledgling disease virus), which infect various parrot species (1-6). APV infections in parrots can cause clinical symptoms in some species (7), inducing chronic disease of the skin and feathers, and frequently, coinfection with beak and feather disease virus (BFDV) (8, 9).

In order to identify APVs circulating in various breeding facilities in Poland, total DNA was extracted from liver samples collected between 2007 and 2011 from 26 deceased parrots (Melopsittacus undulatus, n = 6; Platycercus elegans, n = 2; Psittacula eupatria, n = 1; Psittacula krameri, n = 15; Psittacus erithacus, n = 151; and *Trichoglossus haematodus*, n = 1), as previously described (10–12). Total DNA was enriched by rolling circle amplification using the illustra TempliPhi amplification kit (GE Healthcare, USA), and the concatenated DNA was digested separately with BamH1 and Xmn1 restriction enzymes. The resulting ~5-kb fragments were gel purified and cloned into pJET1.2 (Thermo Fisher) for XmnI-restricted products and pGEM 3Zf(+) (Promega Biotech, USA) for BamHI-restricted products. The cloned products were Sanger sequenced by primer walking at Macrogen, Inc. (South Korea), and the sequence contigs were assembled using the DNA Baser sequence assembler version 4.16 (Heracle BioSoft

SRL, Romania). Of the 26 samples tested, eight birds from three species were found to be positive for APV. They were *P. eupatria* (*n* = 1; PL830), P. krameri (n = 4; PL904, PL1025, PL1220, and PL1233), and *M. undulatus* (n = 3; PL1067, PL1068, and PL1233). The viral genomes were fully sequenced and the analyzed genomewide identity calculated using SDT version 1.2 (13). The genomes share >99.6% identity, while the overall diversity of known APVs (calculated by the inclusion of the 14 genomes available in Gen-Bank) is 0.8%. It is worth noting that six of the eight APV-infected liver samples reported here also contained BFDV (10) and/or parrot hepatitis B virus (PHBV) (11). Two samples, P. eupatria (PL830) and P. krameri (PL1233), were coinfected with both BFDV and PHBV, while PHBV alone had been identified in two additional APV-infected *P. krameri* strains (PL904 and PL1220) (11). BFDV was also previously identified in two M. undulatus strains (PL1067 and PL1068) (10). Neither BFDV nor PHBV was detected in PL1025 (P. krameri) or PL1225 (M. undulatus).

This short communication provides the genome sequences of eight new APVs from three captive parrot species and shows the relatively low diversity of the known APV pool, which so far comprises genome sequences from China, Germany, Japan, and Poland, which have been recovered from various parrot species.

Nucleotide sequence accession numbers. The complete genome sequences have been deposited at GenBank under the accession numbers KT203762 to KT203769.

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REFERENCES

1. Bennett MD, Gillett A. 2014. Butcherbird polyomavirus isolated from a grey butcherbird (*Cracticus torquatus*) in Queensland, Australia. Vet Microbiol 168:302–311. http://dx.doi.org/10.1016/j.vetmic.2013.11.026.

- 2. Guerin JL, Gelfi J, Dubois L, Vuillaume A, Boucraut-Baralon C, Pingret JL. 2000. A novel polyomavirus (goose hemorrhagic polyomavirus) is the agent of hemorrhagic nephritis enteritis of geese. J Virol 74:4523–4529. http://dx.doi.org/10.1128/JVI.74.10.4523-4529.2000.
- Halami MY, Dorrestein GM, Couteel P, Heckel G, Müller H, Johne R. 2010. Whole-genome characterization of a novel polyomavirus detected in fatally diseased canary birds. J Gen Virol 91:3016–3022. http:// dx.doi.org/10.1099/vir.0.023549-0.
- Johne R, Wittig W, Fernández-de-Luco D, Höfle U, Müller H. 2006. Characterization of two novel polyomaviruses of birds by using multiply primed rolling-circle amplification of their genomes. J Virol 80: 3523–3531. http://dx.doi.org/10.1128/JVI.80.7.3523-3531.2006.
- Müller H, Nitschke R. 1986. A polyoma-like virus associated with an acute disease of fledgling budgerigars (*Melopsittacus undulatus*). Med Microbiol Immunol 175:1–13. http://dx.doi.org/10.1007/BF02123124.
- Varsani A, Porzig EL, Jennings S, Kraberger S, Farkas K, Julian L, Massaro M, Ballard G, Ainley DG. 2015. Identification of an avian polyomavirus associated with Adelie penguins (*Pygoscelis adeliae*). J Gen Virol 96:851–857. http://dx.doi.org/10.1099/vir.0.000038.
- Krautwald ME, Muller H, Kaleta EF. 1989. Polyomavirus infection in budgerigars (*Melopsittacus undulatus*)—clinical and etiological studies. Zentralblatt für Veterinärmedizin Reihe B J Vet Med 36:459–467.

- 8. Piasecki T, Wieliczko A. 2010. Detection of beak and feather disease virus and avian polyomavirus DNA in psittacine birds in Poland. Bull Vet Inst Pulawy 54:141–146.
- 9. Ramis A, Latimer KS, Gibert X, Campagnoli R. 1998. A concurrent outbreak of psittacine beak and feather disease virus, and avian polyomavirus infection in budgerigars (*Melopsittacus undulatus*). Avian Pathol 27: 43–50. http://dx.doi.org/10.1080/03079459808419273.
- Julian L, Piasecki T, Chrzastek K, Walters M, Muhire B, Harkins GW, Martin DP, Varsani A. 2013. Extensive recombination detected among beak and feather disease virus isolates from breeding facilities in Poland. J Gen Virol 94:1086–1095. http://dx.doi.org/10.1099/vir.0.050179-0.
- 11. Piasecki T, Harkins GW, Chrząstek K, Julian L, Martin DP, Varsani A. 2013. *Avihepadnavirus* diversity in parrots is comparable to that found amongst all other avian species. Virology 438:98–105. http://dx.doi.org/10.1016/j.virol.2013.01.009.
- 12. Piasecki T, Kurenbach B, Chrząstek K, Bednarek K, Kraberger S, Martin DP, Varsani A. 2012. Molecular characterisation of an avihepadnavirus isolated from *Psittacula krameri* (ring-necked parrot). Arch Virol 157:585–590. http://dx.doi.org/10.1007/s00705-011-1197-3.
- 13. Muhire BM, Varsani A, Martin DP. 2014. SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9:e108277. http://dx.doi.org/10.1371/journal.pone.0108277.