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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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Polyomaviruses (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*, $n = 1$; 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 genome-wide 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 GenBank) 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.
3. **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.
4. **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.
5. **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.
6. **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.
7. **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.
10. **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.