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Identification of Adeno-associated Virus in Muscovy Ducks with Chronic Diarrhea

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ABSTRACT

Adeno-associated viruses (AAVs) are defective members of the genus *Dependoparvovirus*. Waterfowl parvoviruses, another member of the *Dependoparvovirus*, were found to be the closest relative of AAVs. This study was performed to identify the genetic changes that may occur to goose parvovirus (GPV) in one Muscovy duck flock that was observed for 12 weeks after the virus was isolated. Persistent watery diarrhea and wing deformity were the common signs. Cloacal swabs were collected from diseased ducks. Unexpectedly, the identified virus was an AAV. The closest strains were duck AAVs at the nucleotide level, identified in Australia and China. Meanwhile, only 52.3% of nucleotide identity was shared with the GPV strain, previously identified from this flock. Duck adenovirus (DAdV) could not be identified in the samples. This study is one of the first studies in which genetic changes of GPV were tracked. In addition, emerging duck AAV from GPV is suggested, which will be useful for future virus classification.

Keywords: Adeno-associated virus, Chronic diarrhea, Muscovy ducks

INTRODUCTION

Adeno-associated viruses (AAVs) are defective and considered promising therapeutic viral vectors because of their *in vivo* transduction ability with induction of mild immune response and with no evidence of toxicity (Bello et al., 2014; Samulski and Muzyczka 2014; Bennett et al., 2017). Due to their defectiveness, these viruses can not complete their replication cycle except with a helper virus that can be Adenovirus, Herpes virus, Varicella, Cytomegalovirus, or Bocavirus (Georg-Fries et al., 1984; Ni et al., 1994; Wang et al., 2017). In addition to helper viruses, carcinogens and genotoxic agents can render cells permissive to the replication of AAVs (Schlehofer et al., 1986; Yakinoglu et al., 1988; Berns 1990). Without any helper virus, latent infection is established (Berns 1990; Sun et al., 2010; Meier et al., 2020). Viral particles of AAVs consist of a small nonenveloped capsid of about 260 Å in diameter that is composed of three viral proteins: VP1, VP2, and VP3. Their genome is single-stranded DNA of about 4.7-kb with identical inverted terminal repeats at both ends of approximately 150 nucleotides that have genome replication and packaging signals (Bennett et al., 2017; Hildebrandt et al., 2020). Genus *Dependoparvovirus*, a member of the family Parvoviridae, comprises AAVs and the autonomous waterfowl parvoviruses (goose and Muscovy duck parvoviruses, Kailasan et al., 2015; Hildebrandt et al., 2020). Goose parvovirus (GPV) is the causative agent of Derzsy's disease (Derzsy, 1967; Kisary and Derzsy, 1974) that affects Muscovy ducks and geese and is characterized by growth retardation, feathering disorders, and is associated with high mortality rates (Tatár-kis et al., 2004; Glávits et al., 2005). A GPV-related group causes short beak and dwarfism syndrome, and the affected Muscovy ducks show feathering disorders, short beak, tarsus, strong growth retardation, and low morbidity rate (Palya et al., 2009). Muscovy duck parvovirus infects the ducks, causing weakness, locomotor problems, and recumbency (Poonia et al., 2006; Palya, 2020). Duck adeno-associated virus (DAAV) was first identified in clinical samples collected from Muscovy ducks with signs of adenovirus infection in China (Su et al., 2017). Another distantly

related DAAV was identified in fecal samples collected from wild Pacific black ducks in Australia (Vibin et al., 2020). The current study was conducted to re-identify goose parvovirus (GPV) in Muscovy ducks 12 weeks after the first isolation of the virus from these ducks and to determine the genetic changes that may occur due to chronic infection.

MATERIALS AND METHODS

Ethics approval

Samples were collected according to the Animal Care and Biosafety Committee of the Animal Health Research Institute (AHRI 121119).

Samples

Goose parvovirus strain HS1 (accession Number OL763424) was isolated from cloacal swabs obtained from a Muscovy duck flock that consisted of 4 weeks old 60 female and 10 male ducks in Behira governorate, Egypt, in May 2020. These ducks had no vaccination history and suffered from retarded growth, loss of feathers, recumbency, whitish watery diarrhea, and wing deformity. This flock was observed for a study period that extended for 12 weeks post-isolation of GPV. At the end of the study period, cloacal swabs were collected from 15 ducks with chronic diarrhea and wing deformity to determine the possibility of the persistence of GPV infection. Swabs were pooled, suspended in phosphate buffered saline, and centrifuged (Germany) at 8000 × g for 15 minutes. The supernatant was filtered through a 0.22 µm filter and kept in -80°C until the detection of viral DNA by Polymerase chain reaction (PCR).

Viral DNA detection by the polymerase chain reaction

To avoid any genetic mutation that may occur during viral isolation because of viral adaptation to duck embryo or tissue culture, viral DNA was extracted directly from the samples without viral isolation. DNA extraction was performed using QIAamp Mini Elute Virus Kit (Qiagen, Germany) following the manufacturer's instructions. Extracted DNA was amplified using a buffer mix (Emerald Amp Max, Takara, Japan) and primers listed in Table 1. Primers directed to partially amplify the VP1 gene of GPV were used based on the previous history of the infection, whereas DAAV was detected unintentionally with the same primers but with different sizes of amplified sequences. Due to the identification of DAAV in the samples, another set of primers was used to detect duck adenovirus (DAdV). The PCR was performed using Biometra T3000 thermocycler (Biometra, Germany). Electrophoresis was done through 1.5% agarose gel and a 100 bp DNA marker (Thermo Scientific, USA).

Table 1. Primers used in the study

Virus	Primers	References
Goose parvovirus Duck adeno-associated virus	F (5'-CCTGGCTATAAGTATCTTGG-3') R (5'-GTAGATGTGGTTGTTGTAGC-3')	Poonia et al., 2006
Duck adenovirus	F (5'-CACTCACGGGAACTG-3') R (5'-GGGCACCACAAACG-3')	Zhang et al., 2016

DNA sequencing

A single 609 bp DNA band was purified using a QIAquick gel extraction kit (Qiagen, USA) and sequenced in both directions with the same primers indicated in Table 1. The sequencing reaction was done using the Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, USA) in ABI automated sequencer (Applied Biosystems 3500xl genetic analyzer, USA).

Phylogenetic analysis

The obtained partial sequences of VP1 were subjected to a basic local alignment search tool (BLAST) within GenBank to determine the closely related strains. Nucleotide sequences and their deduced amino acids were aligned using CLUSTALW with 1000 bootstrap replications in BioEdit software (version 7.2.5). The maximum composite likelihood method with 1000 bootstrap replications of MEGA11 software was used to determine pairwise distance and to construct a neighbor-joining (NJ) tree (Tamura et al., 2021).

RESULTS AND DISCUSSION

Adeno-associated viruses share the same genome organization as waterfowl parvoviruses (Zadori et al., 1995). Moreover, at the level of amino acid sequence, waterfowl parvoviruses were found to be the closest relative of adeno-associated virus 2. Thus, GPV was reclassified with AAV under the genus

Dependoparvovirus (Brown et al., 1995; Zadori et al., 1995).

Goose parvovirus was isolated and identified from a Muscovy duck flock with retarded growth, feather disorders, diarrhea, and wing deformity. During this study, this flock was observed for 12 weeks after GPV was isolated to determine the persistent possibility of clinical signs associated with GPV infection. During this period mortality rate was 30%, and the common signs were chronic persistent watery diarrhea and wing deformity, which were reported in about 80% of the affected flock. Therefore, cloacal swabs were collected as pooled samples from ducks that suffered from these symptoms to re-identify GPV in this flock. The identified virus was AAV, not GPV. Both sites of the primers used in the detection of GPV (Table 1) were similar to the analogous sites at the identified DAAV (with only two nucleotide differences in forwarding primer), which enabled its unintended detection. Based on the partially DNA sequenced VP1, the identified virus designated HSCH (accession number. ON166703) shared only 52.3% and 49.1% nucleotide and amino acid identities with the GPV (HS1), respectively, which was previously isolated from this flock (Table 2). This DNA sequence which includes the N terminus of VP1, 2, and 3, was found to be 15 bases longer than that of GPV due to many nucleotides' insertions.

The closest strain was PBDAAV/PBD12, which was identified from wild ducks in Australia with 87.4% and 97.4% nucleotide and amino acid identities, respectively (Vibin et al., 2020). Phylogenetic analysis revealed separate clustering of the identified strain within avian *Dependoparvoviruses*, including autonomous waterfowl parvoviruses, DAAVs, and avian AAVs (AAAVs), which all shared the same ancestor (Figure 1). In addition, the identified strain possessed 18 unique amino acid substitutions, most of which were located at the N terminus of VP2 and 3 (Figure 2). A nucleotide identity of 82.7% was found with DAAV (MHH-05-2015) that was also identified in Muscovy duck in China (Su et al., 2017). DAAV (MHH-05-2015) virus was found to be dependent on DAdV infection. Therefore, an attempt was made to detect co-infection with DAdV. The DAdV could not be identified in the samples. Adeno-associated viruses have dependent replication nature as they need another helper virus, carcinogen, or genotoxic agent to complete their replication cycle (Schlehofer et al., 1986; Yakinoglu et al., 1988; Ni et al., 1994; Wang et al., 2017). Accordingly, it can be suggested that this flock may be affected by a helper agent other than DAdV. Another possible explanation for the absence of DAdV from the sample is the emergence of the identified duck AAV from GPV strain HS1, which was previously identified from the same flock. Hildebrandt et al. (2020) have done phylogeny reconstruction of endogenous viral elements of *Dependoparvoviruses* (viral genetic remnants integrated into host genomes for millions of years) and suggested the autonomous highly pathogenic exogenous *Dependoparvovirus* ancestors of these elements that co-evolved with waterfowl birds. This finding and the persistence of diarrhea since GPV infection support the suggestion of the emergence of DAdV from GPV. This was also supported by Kailasan et al., 2015 who reported that parvoviruses had high evolution rates similar to RNA viruses, and cell passages with very small numbers could induce the selection of new natural mutants.

Table 2. Nucleotide and amino acid identities with other goose parvoviruses and duck adeno-associated viruses identified in Muscovy ducks

	Amino acids identities									
Strains	1	2	3	4	5	6	7	8	9	10
1-M15		98.5	96.4	97.4	79.3	98	49.9	50.8	49.9	50.8
2- JS1603	98.6		95.9	96.9	79.9	97.4	49.9	50.8	49.9	50.8
3- LH	95.4	95.6		99	79.3	95.3	50.8	51.6	51.6	52.4
4- B	96.7	97.1	97.6		80.5	96.4	51.6	52.4	51.6	53.2
5- FM	77.5	78.3	78	78.6		78.7	45.7	49.1	49.1	49.1
6- HS1	97.6	97.4	96	96.9	77.8		49.1	49.1	48.2	49.1
7- HSCH	51.3	52	55	52.6	53.4	52.3		85.3	84.8	85.9
8- PBDAAV/PBD12	54.1	54.7	56.5	54.4	59	54	87.4		97.4	87.1
9- DAAV/G002-20	54	54.6	56.4	55	60.2	54.4	86.5	97.9		85.9
10- MHH-05	50.9	51.7	53.7	52.2	56.9	50.6	82.7	80.4	80.4	
	Nucleotides identities									

The strain that was identified during this study is highlighted

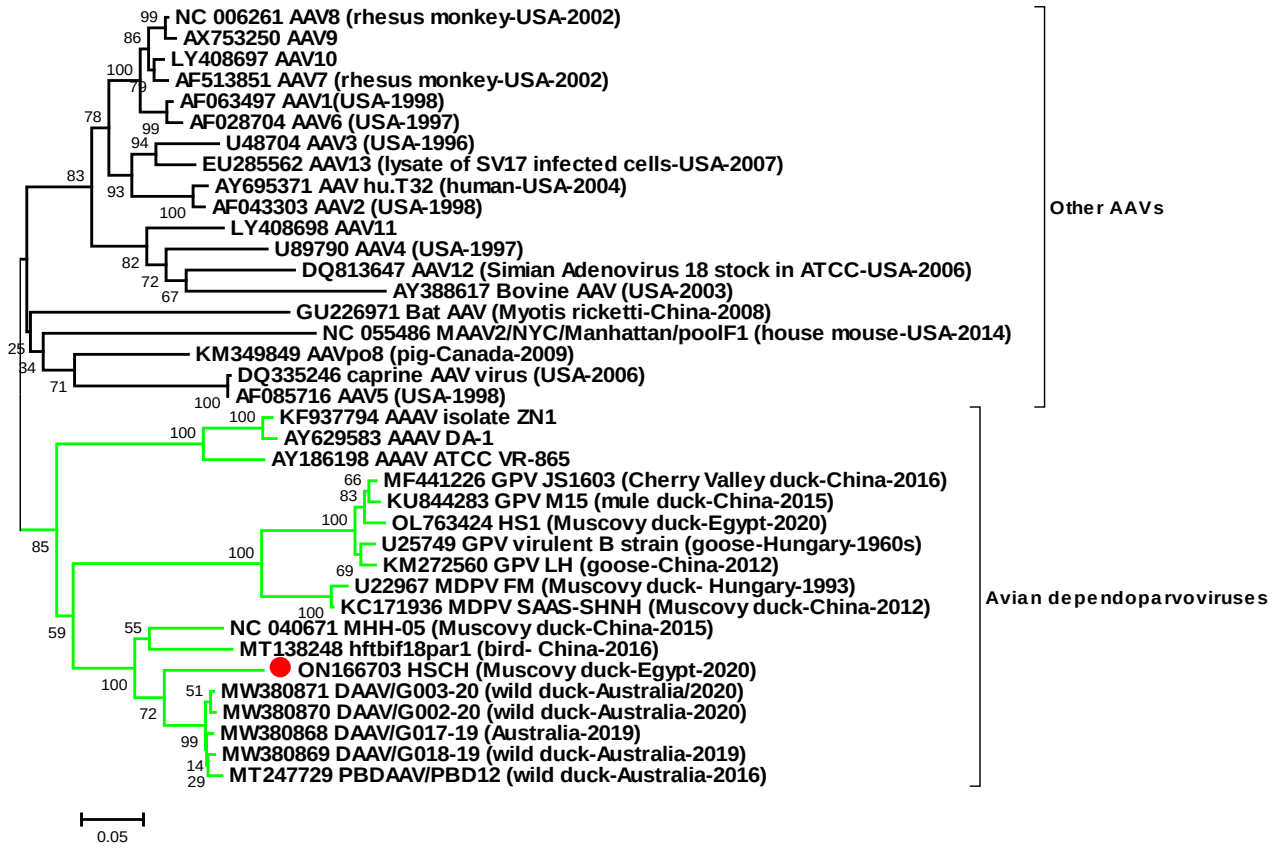

Figure 1. Neighbor-joining tree indicates clustering of the identified strain separately within avian *Dependoparvoviruses* (autonomous waterfowl parvoviruses and AAAVs) identified in Muscovy ducks. This tree shows a common ancestor with avian *Dependoparvoviruses*. Green branches indicate avian *Dependoparvoviruses* while black branches indicate AAVs other than avian origin. The red circle indicates the identified strain.

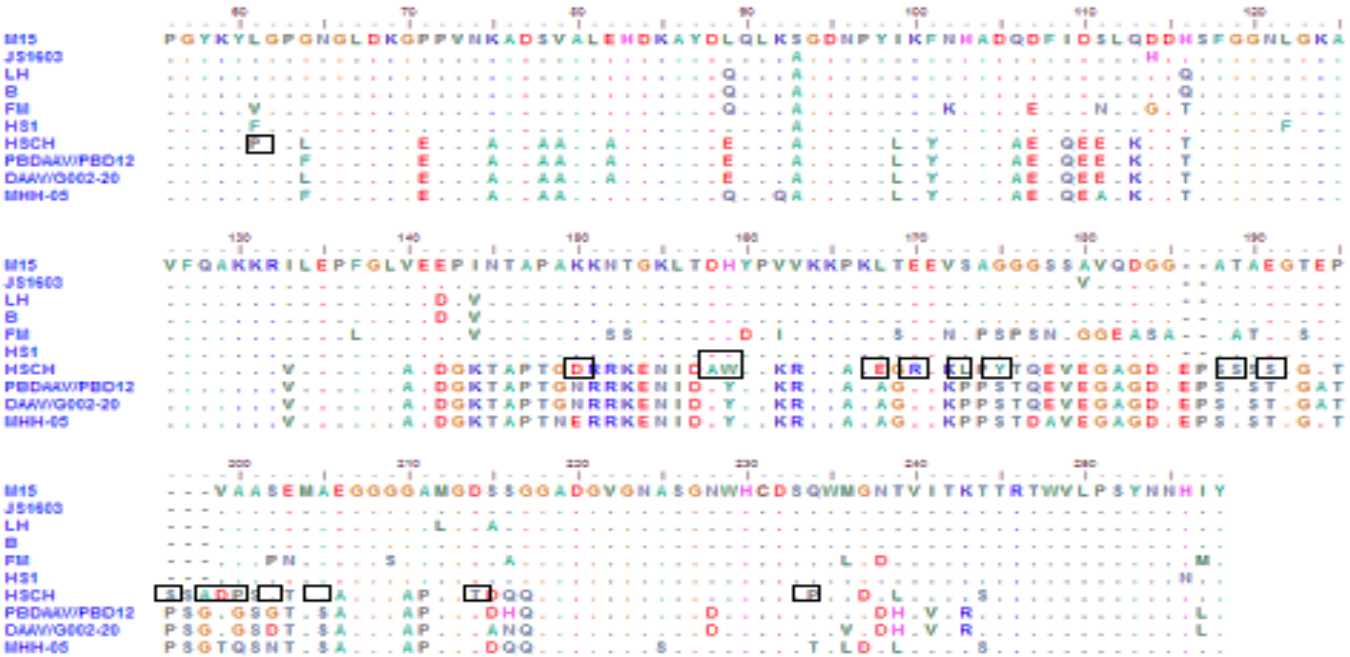

Figure 2. Amino acids substitutions with autonomous *Dependoparvoviruses* and closely related duck adeno-associated viruses

This study is one of the first identification of DAAV from Muscovy ducks in Egypt. Moreover, it indicates the possibility of emerging DAAV from GPV. Further study should be done experimentally to confirm the possibility of emerging DAAV from GPV to understand the pattern of viral evolution better.

DECLARATIONS

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This study was done without external funding.

Authors' contribution

Hamdi Mohamed Sallam designed the study, collected the samples, carried out the molecular genetic study, and wrote the manuscript. Ali Mahmoud Zanaty performed DNA sequencing and revised the manuscript. All authors checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors declare that they have no competing interests.

Ethical consideration

All ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all authors.

REFERENCES

Bello A, Chand A, Aviles J, Soule G, Auricchio A, and Kobinger GP (2014). Novel adeno-associated viruses derived from pig tissues transduce most major organs in mice. Scientific Reports, 4: 6644. DOI: https://www.doi.org/10.1038/srep06644

Bennett A, Mietzsch M, and Agbandje-McKenna M (2017). Understanding capsid assembly and genome packaging for adeno-associated viruses. Future Virology, 12(6): 283-297. DOI: https://www.doi.org/10.2217/fvl-2017-0011

Berns KI (1990). Parvovirus replication. Microbiology and Molecular Biology Reviews, 54(3): 316-329. DOI: https://www.doi.org/10.1128/mr.54.3.316-329.1990

Brown KE, Green SW, and Young NS (1995). Goose parvovirus-an autonomous member of the Dependovirusgenus?. Virology, 210(2): 283-291. DOI: https://www.doi.org/10.1006/viro.1995.1345

Derzsy D (1967). A viral disease of goslings I. Epidemiological, clinical, pathological, and aetiological studies. Acta veterinaria Academiae Scientiarum Hungaricae, 17(4): 443-448. Available at: https://pubmed.ncbi.nlm.nih.gov/5626296/

Georg-Fries B, Biederlack S, Wolf J, and Zur Hausen H (1984). Analysis of proteins, helper dependence, and seroepidemiology of a new human parvovirus. Virology, 134(1): 64-71. DOI: https://www.doi.org/10.1016/0042-6822(84)90272-1

Glávits R, Zolnai A, Szabó E, Ivanics E, Zarka P, Mató T, and Palya V (2005). Comparative pathological studies on domestic geese (*Anseranser domestica*) and Muscovy ducks (*Cairinamoschata*) experimentally infected with parvovirus strains of goose and Muscovy duck origin. Acta Veterinaria Hungarica, 53(1): 73-89. DOI: https://www.doi.org/10.1556/AVet.53.2005.1.8

Hildebrandt E, Penzes JJ, Gifford RJ, Agbandje-Mckenna M, and Kotin RM (2020). Evolution of *Dependoparvoviruses* across geological timescales—implications for design of AAV-based gene therapy vectors. Virus Evolution, 6(2): veaa043. DOI: https://www.doi.org/10.1093/ve/veaa043

Kailasan S, Agbandje-McKenna M, and Parrish CR (2015). Parvovirus family conundrum: What makes a killer?. Annual Review of Virology, 2(1): 425-250. DOI: https://www.doi.org/10.1146/annurev-virology-100114-055150

Kisary J and Derzsy D (1974). Viral disease of goslings IV. Characterization of the causal agent in tissue culture system. Acta Veterinaria Academiae Scientiarium Hungaricae, 24: 287-292. Available at: https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL7534010275

Meier AF, Fraefel C, and Seyffert M (2020). The interplay between adeno-associated virus and its helper viruses. Viruses, 12(6): 662. DOI: https://www.doi.org/10.3390/v12060662

Ni T, Zhou X, McCarty DM, Zolotukhin I, and Muzyczka N (1994). *In vitro* replication of adeno-associated virus DNA. Journal of Virology, 68(2): 1128-1138. DOI: https://www.doi.org/10.1128/jvi.68.2.1128-1138.1994

Palya V, Zolnai A, Benyeda Z, Kova´cs E, Kardi V, and Mato´ T (2009). Short beak and dwarfism syndrome of mule duck is caused by a distinct lineage of goose parvovirus. Avian Pathology, 38(2): 175-180. DOI: https://www.doi.org/10.1080/03079450902737839

Palya VJ (2020). Parvovirus infections of waterfowl. In: DE. Swayne (Editor), Diseases of poultry, 14th Edition. Hoboken, NJ: Wiley-Blackwell. pp. 474-497. Available at: https://books.google.com/books?hl=en&lr=&id=WWsUAAAAQBAJ&oi=fnd&pg=PA444&dq=Palya+VJ+(2020).+Parvovirus+infections+of+waterfowl&ots=3eM0hoDYDJ&sig=ttE_fxTVGok6DceIHPDBEQdPCgM#v=onepage&q&f=false

Poonia B, Dunn PA, Lu H, Jarosinski KW, and Schat KA (2006). Isolation and molecular characterization of a new Muscovy duck parvovirus from Muscovy ducks in the USA. Avian Pathology, 35(6): 435-441. DOI: https://www.doi.org/10.1080/03079450601009563

Samulski RJ and Muzyczka (2014). AAV-mediated gene therapy for research and therapeutic purposes. Annual Review of Virology, 1: 427-451. DOI: https://www.doi.org/10.1146/annurev-virology-031413-085355

Schlehofer JR, Ehrbar M, and zur Hausen H (1986). Vaccinia virus, herpes simplex virus and carcinogens induce DNA amplification in a human cell line and support replication of a helper virus-dependent parvovirus. Virology, 152(1): 110-117. DOI: https://www.doi.org/10.1016/0042-6822(86)90376-4

Su XN, Liu JJ, Zhou QF, Zhang XH, Zhao LC, Xie QM, Chen WG, and Chen F (2017). Isolation and genetic characterization of a novel adeno-associated virus from Muscovy ducks in China. Poultry Science, 96(11): 3867-3871. DOI: https://www.doi.org/10.3382/ps/pex235

Sun X, Lu Y, Bish LT, Calcedo R, Wilson JM, and Gao G (2010). Molecular analysis of vector genome structures after liver transduction by conventional and self-complementary adeno-associated viral serotype vectors in murine and nonhuman primate models. Human Gene Therapy, 21(6): 750-761. DOI: https://www.doi.org/10.1089/hum.2009.214

Tamura K, Stecher G, and Kumar S (2021). MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38(7): 3022-3027. DOI: https://www.doi.org/10.1093/molbev/msw054

Tatár-kis T, Mató T, Markos B, and Palya V (2004). Phylogenetic analysis of Hungarian goose parvovirus isolates and vaccine strains. Avian Pathology, 33(4): 438-444. DOI: https://www.doi.org/10.1080/03079450410001724067

Vibin J, Chamings A, Klaassen M, Raj Bhatta T, and Alexandersen S (2020). Metagenomic characterization of avian parvoviruses and picornaviruses from Australian wild ducks. Scientific Reports, 10: 12800. DOI: https://www.doi.org/10.1038/s41598-020-69557-z

Wang Z, Deng X, Zou W, Engelhardt JF, Yan Z, and Qiu J (2017). Human bocavirus 1 is a novel helper for adeno-associated virus replication. Journal of Virology, 91(18): e00710-17. DOI: https://www.doi.org/10.1128/JVI.00710-17

Yakinoglu AO, Heilbronn R, Burkle A, Schlehofer JR, and zur Hausen H (1988). DNA amplification of adeno-associated virus as a response to cellular genotoxic stress. Cancer Research, 48(11): 3123-3129. Available at: https://aacrjournals.org/cancerres/article/48/11/3123/492704/DNA-Amplification-of-Adeno-associated-Virus-as-a

Zadori Z, Stefancsik R, Rauch T, and Kisary J (1995). Analysis of the complete nucleotide sequences of goose and Muscovy duck parvoviruses indicates common ancestral origin with adeno-associated virus 2. Virology, 212(2): 562-573. DOI: https://www.doi.org/10.1006/viro.1995.1514

Zhang X, Zhong Y, Zhou Z, Liu Y, Zhang H, Chen F, Chen W, and Xie Q (2016). Molecular characterization, phylogeny analysis and pathogenicity of a Muscovy duck adenovirus strain isolated in China in 2014. Virology, 493: 12-21. DOI: https://www.doi.org/10.1016/j.virol.2016.03.004