**First report of a novel duck astrovirus causing gout disease in ducklings**

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**CONFLICT OF INTEREST**

The authors declare no conflicts of interest with respect to the research, authorship, and publication of this article.

**Summary**

Four divergent groups of duck astroviruses (DAstVs) have been identified that infect domestic ducks. In March 2021, a fatal disease characterized by visceral urate deposition broke out in 5-day-old Beijing ducks on a commercial farm in Guangdong province, China. The pathogen was confirmed to be a duck astrovirus. The complete genome sequence of this DAstV was obtained by virome sequencing and amplification. Phylogenetic analyses and pairwise comparisons demonstrated that this DAstV represented a novel group of avastrovirus. Thus, we designated this duck astrovirus as DAstV-5 JM strain. DAstV-5 JM shared genome sequence identities of 15–45% with other avastroviruses. Amino acid identities with proteins from other avastroviruses did not exceed 59% for ORF1a, 79% for ORF1b, and 60% for ORF2. The capsid region of JM shared genetic distances of 0.596 to 0.695 with the three official avastrovirus species. In summary, we determined that the DAstV-5 JM strain, causing gout in ducklings, is a novel species of avastrovirus.

**KEYWORDS**

Duck astrovirus, gout, genome sequence, phylogenetic analysis, genetic distance

**1 INTRODUCTION**

Astroviruses (AstVs) belong to the *Astroviridae* family and are infectious, non-enveloped, positive sense, single-stranded RNA viruses. Astroviruses infect a wide variety of hosts, including humans, sheep, dogs, cats, mink, chickens, ducks, geese, guinea fowl, and turkeys. According to their host range, the *Astroviridae* family is divided into two genera: *Mamastrovirus* and *Avastrovirus*. The two genera comprises 22 species according to the International Committee on Taxonomy of Viruses (ICTV), namely *Mamastroviruses* 1–19 and *Avastroviruses* 1–3 (ICTV 2011). Astroviruses not only pose a great threat to human health and the breeding industry, but also have the potential for cross-species transmission (Roach and Langlois 2021). The 19 species of *Mamastroviruses* mainlycause diseases that are closely related to gastroenteritis and encephalitis in mammals (Cordey et al. 2016; Küchler et al. 2019). The three *Avastroviruses* include *Avastrovirus 1* (turkey astrovirus 1), *Avastrovirus 2* (avian nephritis virus 1 and avian nephritis virus 2)and *Avastrovirus 3* (turkey astrovirus 2 and duck astrovirus 1).These *Avastroviruses* are tightly linked to avian diseases, such as hepatitis (Yugo et al. 2016), enteritis (Nuñez et al. 2018; Jindal et al. 2011), runting-stunting syndrome (Kang et al. 2018; Kim et al. 2020), white chicken syndrome (McIlwaine et al. 2021), and gout (Zhang et al. 2021). Besides, there are many other newly discovered species of astroviruses that have not been classified, but have been proposed to the ICTV.

To date, chicken astrovirus and goose astrovirus have been found to cause avian gout diseases. Chicken astrovirus was reported as the causative agent of broiler chicken gout for the first time in India in 2013 (Bulbule et al. 2013a). Another study revealed the presence of chicken astrovirus in broiler chickens affected with gout in north-western India in 2019 (Panigrahi et al. 2019). Recently, chicken astrovirus was also been confirmed to be the pathogenic agent of gout in Malaysian chicken (Raji et al. 2021). Goose astrovirus was observed to cause fatal visceral gout in domestic goslings in China in 2017 (Zhang et al. 2018). Goose astroviruses isolated from ducks, such as the SDXT, SDTA and HNNY0620 strains, could also cause gout in ducklings (Chen, Zhang, et al. 2020; Wei et al. 2020; Chen et al. 2021). In addition, goose astrovirus has been validated to cause gout in experimentally infected chickens (Li et al. 2021). However, no case of duck astrovirus-induced gout in ducks has been reported.

To date, four groups of duck astroviruses have been identified: duck astrovirus 1 (DAstV-1), duck astrovirus 2 (DAstV-2), duck astrovirus 3 (DAstV-3), and duck astrovirus 4 (DAstV-4). These four duck astroviruses are genetically different from each other. DAstV-1 and DAstV-2, also known as duck hepatitis 2 (DHV-2) and duck hepatitis 3 (DHV-3), cause hepatitis in ducklings (Yugo et al. 2016). DAstV-3 was first found in newly hatched, healthy Pekin ducklings in 2013 (Liu, Wang, and Zhang 2014). DAstV-4 was isolated from Pekin ducks in live-bird markets in Guangdong province (Liao et al. 2015). However, no disease has been reported to be caused by DAstV-3 and DAstV-4.

In the present study, we discovered a new duck astrovirus JM strain, namely DAstV-5, which caused visceral gout in Beijing ducks on a duck farm in Jiangmen, Guangdong province. We conducted virome sequencing and obtained the complete genome sequence of the virus. Phylogenetic analyses and pairwise comparisons demonstrated that this virus was different from all the other previously known *Avastroviruses*, and thus represents a novel species of duck astrovirus.

1. **MATERIALS AND METHODS**

**2.1 Bacteriological and virological examinations**

For bacteriological diagnosis, kidney and liver tissues were collected from dead ducklings and inoculated onto tryptic soy agar plates containing 2% fetal bovine serum (Hyclone, Shanghai, China) immediately. The plates were incubated at 37 ℃ with 5% CO2 for 48 h. For virological detection, kidney and liver tissue samples were homogenized in phosphate-buffered saline (PBS) and pooled together for three cycles of freezing and thawing. The suspensions were centrifuged at 12,000 × *g* for 10 minutes and then the supernatant was collected for virus RNA/DNA extraction using a Tianlong Nucleic Acid Extraction & Purification Kit T180H (Tianlong Technology Co., Ltd., Shaanxi China). The extracted RNA/DNA was subjected to PCR amplification to detect goose astrovirus (GAstV), avian orthoreovirus virus (ARV), duck Tembusu virus (DTMUV), novel duck parvovirus (NDPV), duck circovirus (DCV), goose parvovirus (GPV), duck enteritis virus (DEV), duck adenovirus (DAdV), avian influenza virus (AIV), and Newcastle disease virus (NDV). The primers used to amplify the genome sequences were detailed to our previous research (Huang et al. 2020; Huang et al. 2021) and are shown in Table S1.

* 1. **Sample processing**

Five grams liver tissue was subjected to grinding, added with 5 volumes of precooled sterile stabilization buffer, and vortexed for 5 minutes. After three rounds of freeze-thawing, the sample was centrifuged at 12000 × *g* for 5 minutes to remove the precipitate. Cell fragments were removed by filtering through 0.45 um + 0.22 um filter membrane. The supernatant was transferred to an ultracentrifugation tube containing 28% (w / w) sucrose. The sample was centrifuged at 160000 × *g* for 2 h at 4 °C in an. HIMAC CP 100wx ultracentrifuge (Hitachi, Tokyo, Japan). After removing the supernatant, the pellet was resuspended in 200 μl SB buffer. Enzyme mix and enzyme mix buffer were added proportionally and the samples were incubated at 37 °C for 60 min. Stop solution solution (2 μl) was then added proportionally, mixed well, and incubated at 65–75 °C for 10 minutes to inactivate the enzyme reaction. The samples were centrifuged at 2000 × g for 5 minutes, and 200 μl of the supernatant was at −20 °C for subsequent experiments.

* 1. **Virome sequencing and analysis**

The supernatant was used to extract DNA/RNA with an R6662-02 MagPure Viral DNA/RNA Mini LQ Kit (R6662-02, Angen Biotech, Guangzhou, China). The whole genome was amplified using REPLI-g Cell WGA & WTA Kit (150054, Qiagen, Germantown, MD, USA). Sequencing libraries were generated using an NEB Next® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations and index codes were added. The library quality was assessed using a Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY, USA) and Agilent 4200 (Agilent, Santa Clara, CA, USA) system. Finally, the library was sequenced on an Illumina Novaseq 6000 sequencer (Illumina Inc., San Diego, CA, USA) and 150 bp paired-end reads were generated. The raw data were processed using SOAPnuke (v1.5.6) to acquire the clean data for subsequent analysis (Chen et al. 2018).

We used BLAST software (v2.9.0 +; NIH, Bethesda, MD, USA) to compare the unique contigs with virus database (separated from the NT database). If the alignment similarity was ≥ 80%, the alignment length was ≥ 500 bp, and the e-value was ≤ 1e-5, the sequence was defined as a virus sequence. If the alignment length was ≥ 100 bp, and the e-value was ≤ 1e-5, the sequence was defined as suspected virus sequence. After comparing contigs with the virus database separated from the NT database and the virus database separated from the NR and HMM (vpfs and vfam) databases, we obtained the union as the candidate virus sequence. Then, the candidate virus sequences were compared with NCBI taxonomy database. If more than 20% of the first 50 alignment results supported the fact that the sequences were non-viral sequences, the candidate virus sequences would be excluded and the rest would be considered as new virus sequences. The virome sequencing and data analysis were performed by Magigene Biotechnology Corporation (Guangzhou, China)

* 1. **Obtaining complete genome sequence of the virus**

Four pairs of PCR primers were designed to amplify the gaps (Table S2) using 2 × Taq Plus Master Mix (Vazyme, Jiangsu, China). The four amplified segments were ligated to the pGEM-T vector and sequenced. The 5' and 3' untranslated regions (UTRs) were obtained using 5' and 3' rapid amplification of cDNA ends (RACE) as described previously (Liu et al. 2014). To verify the complete genome sequence, we designed eight pairs of primers. The primers used to amplify the genome sequences are shown in Table S2. The complete genome sequence was assembled by DNAMAN 6.0.3 (Lynnon Biosoft, San Ramon, CA, USA).

* 1. **Sequence analysis**

Transmembrane (TM) domains and functional proteins were predicted using the online Sample Modular Architecture Research Tool (SMART) analysis tool (<http://smart.embl-heidelberg.de/>). The stem-loop II-like motif (s2m) was evaluated using the online Rfam analysis tool (http:/rfam.xfam.org). The open reading frames (ORFs) were analyzed by DNAMAN version 8.0 (Lynnon Biosoft). Phylogenetic trees for the complete genome and the deduced amino acid sequences of the three open reading frames (ORFs) were constructed in the MEGA 7.0 software using the neighbor-joining method, with 1000 bootstrap replications (Kumar, Stecher, and Tamura 2016). Amino acid genetic distances were analyzed using the *p*-dist method of MEGA 7.0 software, using pairwise deletion treatment. Bootstrap analysis was performed with 100 replications. Pairwise comparisons of DAstV-5 JM with other avastroviruses (Turkey astrovirus 1, Avian nephritis virus 1, Turkey astrovirus 2, Duck astrovirus 1-4, Chicken astrovirus, and Goose astrovirus) were conducted using the MegAlign 7.1.0 software (DNASTAR Inc., Madison WI, USA) employing the Clustal W method.

* 1. **Genetic recombination analysis**

Recombination event analysis of DAstV-5 JM with other astroviruses (including *Mamastroviruses* and *Avastrovirus*) was evaluated using Recombination Detection Program (RDP) software Version 4.101 with the RDP, GENECONV, Bootscan, MaxChi, Chimaera, SiScan, and 3Seq methods (Martin et al. 2015).

1. **RESULTS** 
   1. **Case history and microbiological examination**

In March 2021, a gout disease broke out in 5-day-old Beijing ducks on a commercial farm in Guangdong province, China. The mortality rate was 20% on this farm. At necropsy, urate deposits were observed mainly in the heart, mesenterium, and gallbladder of the dead ducklings (Figure 1). The liver and heart tissues of the dead ducklings were collected for bacteriological and virological examinations. Bacteriological examination was conducted as described previously (Zhang et al. 2021), and no pathogenic bacteria could be isolated from the diseased ducklings. The DNA/RNA were extracted from the liver and heart tissues and the isolated nucleic acids tested negative for GAstV, ARV, DTMUV, NDPV, DCV, GPV, DEV, DAdV, AIV, and NDV by PCR assays.

* 1. **Complete genome sequence of DAstV-5 JM strain**

To identify the pathogen, we carried out virome sequencing and analysis. Five astrovirus segments were identified. The segments were 253 bp to 1006 bp in length. Comparison with previously published nucleotide sequences show that the homology was 52–77% (Table 1). The presence of astrovirus (designated JM) was confirmed by amplification of a 782 bp sequence (data not shown).

To obtain the whole genome sequence of this duck astrovirus JM strain, we amplified and sequenced the four gaps of the five detected sequences. The 5' and 3' UTRs were obtained using 5' and 3' RACE. Then, the sequences of five segments, four gaps, and two UTRs were ligated together. Finally, we obtained a 7517 bp viral genome sequence of JM strain. The ligated whole genome sequence was verified by PCR amplification and sequencing. Alignment results showed that the amplified sequences were in accordance with the ligated whole genome sequence. The complete genomic sequence of the virus, named as DAstV-5 JM strain, has been submitted to the GenBank database under the accession number OM095382.

* 1. **Genetic characteristics of the DAstV-5 JM strain**

The predicted genomic organization of DAstV-5 JM is shown in Figure 2. The genomic length of DAstV-5 JM is 7517 nt, including a 5' UTR (1–105), ORF1a (106–3474), ORF1b (3465–5015), ORF2 (5041–7278), 3' UTR (7279–7498), and poly (A) tail (7499–7517). ORF1a comprises 3369 nt, encoding a polypeptide of 1122 aa, with a calculated molecular weight of 128.62 kDa. ORF1b comprises 1551 nt, encoding a polypeptide of 427 aa, with a calculated molecular weight of 50.27 kDa. ORF2 comprises 2263 nt, encoding a polypeptide of 745 aa, with a calculated molecular weight of 82.42 kDa.

ORF1a and the ORF1b encode the non-structural proteins. ORF1a is not in the same reading frame with ORF1b. As predicted by the online SMART analysis tool, the ORF1a protein contains four transmembrane (TM) domains (aa 395–417, 424–443, 453–475 and 484–506), a trypsin-like serine protease (aa 542–705), and two nuclear localization signal (NLS) motifs (aa 783–797, 895–908).

ORF1b was predicted to encode an RNA-dependent RNA polymerase (RdRp). It overlaps by 10 nt with ORF1a. It starts with a ribosomal frameshift signal containing the heptameric AAAAAAC sequence (3465–3471) and contains a stem loop structure sequence (3478–3501).

ORF2 encodes the capsid protein. The start codon of ORF2 is 25 nt downstream of the stop codon of ORF1b; therefore, ORF2 of DAstV-5 JM is not in the same reading frame as ORF1b. A CCGAA motif (5027–5031) was identified within the 25 nt sequence. Like other astroviruses, a stem-loop, s2m, is also present at the end of ORF2 and the adjacent 3' UTR (7255–7297). Taken together, these results indicated that DAstV-5 JM possesses typical astrovirus features.

* 1. **Phylogenetic analyses and sequence comparisons of the JM strain**

To determine the phylogenetic relationships of duck astrovirus JM strain with other *Avastroviruses*, we conducted phylogenetic analysis of the complete genome of JM and the amino acid sequences of ORF1a, ORF1b, and ORF2. In the complete genome and the ORF1a amino acid phylogenetic trees, DAstV-5 JM strain was most closely related to duck astrovirus CPH (Figure 3 and Figure 4A). In the ORF1b and ORF2 amino acid phylogenetic trees, the DAstV-5 JM strain was closely related to both duck astrovirus CPH and the chicken astrovirus clades (Figure 4B and 4C). Meanwhile, the DAstV-5 JM strain had an outgroup relationship with the duck astrovirus CPH and the chicken astrovirus clades in the ORF1b and ORF2 amino acid phylogenetic trees.

Pairwise comparisons were performed to determine the sequence identities. The results showed that DAstV‑5 JM shared identities of 15–45% in the complete genome sequences with the representative members of recognized and unassigned species in the genus *Avastrovirus* (Table 2). The sequence identities at the amino acid level were 26–59% (ORF1a), 55–79% (ORF1b), and 24–60% (ORF2). AlthoughDAstV-5 JM is most closely related to duck astrovirus CPH, they shared relatively low amino acid identities of 59% (ORF1a), 77% (ORF1b), and 60% (ORF2). According to the species demarcation criteria of the *Avastrovirus* genus from the ICTV, the mean amino acid genetic distances (*p*-dist) should range between 0.576 to 0.742, and 0.204 to 0.284 between and within groups, respectively (Bosch et al. 2011). The results showed that the capsid region of JM shared a genetic distance of 0.596 to 0.695 with the representative strains of *Avastrovirus* 1–3. DAstV-5 JM shared a genetic distance of 0.586 to 0.662 with chicken astrovirus 1(Poland/G059/2014 strain) and goose astroviruses (SDXT, FLX and HNIG strains). DAstV-5 JM shared genetic distances of 0.402, 0.440, and 0.524 with duck astrovirus CPH, chicken astrovirus GA2011, andduck astrovirus YP2, respectively. Therefore, DAstV-5 JM could be identified as a novel Avastrovirus.

* 1. **Genetic recombination analysis**

To detect the possibility of recombination events in DAstV-5 JM strain, we conducted genomic recombinant detection using RDP version 4.101 software. The analysis identified no recombination events between the JM strain and the other avian and mammalian astroviruses using the RDP, GENECONV, Bootscan, MaxChi, Chimaera, SiScan, and 3Seq methods.

**4 DISCUSSION**

Astroviruses are pathogenic to both humans and animals and have been detected from over 80 avian and mammalian host species (Mendenhall, Smith, and Vijaykrishna 2015). Astrovirus-induced gout disease has been prevalent in geese, chickens, and ducks in recent years, resulting in serious economic loses to the poultry industry (Yang et al. 2018; Wei et al. 2020; Chen, Zhang, et al. 2020; Niu et al. 2018; Bulbule et al. 2013b; Sumitra et al. 2019; Raji et al. 2021). In this research, we identified a novel duck astrovirus, namely DAstV-5 JM, which caused the first recorded outbreak of deadly gout in ducklings.

According to the genetic organization analysis, the DAstV-5 JM strain possesses typical genomic characteristics: threes ORFs, TM domains, serine protease, NLS, ribosomal frameshift signal, and RdRp. Meanwhile, DAstV-5 JM strain has its own features. As in the DAstV-4 YP2 strain, DAstV-5 JM ORF1a is in the same reading frame as ORF2; however, ORF1b is not in the same reading frame with ORF1a and ORF2 (Liao et al. 2015). The ORF arrangements are quite different in DAstV‑1, DAstV-2, and DAstV-3. In DAstV-1 (C-NGB strain) and DAstV-3 (CPH strain), all the three ORFs are in different reading frames (Fu et al. 2009; Liu, Wang, and Zhang 2014). In DAstV-2 (SL1 strain), ORF1b is in the same reading frame with ORF2; but ORF1a is not in the same reading frame with ORF1b and ORF2 (Liu et al. 2014). Avastroviruses possess four to six TM domains. DAstV-5 possesses four TM domains in ORF1a, as does DAstV-2, DAstV-3, and DAstV-4. However, DAstV-1 C-NGB strain, GAstV FLX strain, and GAstV HNNY0620 strain possess five TM domains (Fu et al. 2009; Zhang et al. 2017; Chen et al. 2021). ANV-1 (ANV/CHN/BJCP510-2/2018) and CAstV-A (Poland/G059/2014) have six TM domains (Xue et al. 2020; Sajewicz-Krukowska and Domanska-Blicharz 2016). It appears that the number of TM domain is not related to the species of astrovirus. However, the function of the TM domains in astroviruses requires further exploration.

The DAstV-5 JM strain contains one s2m element at the end of ORF2 and the adjacent 3' UTR. Most astroviruses contains only one s2m element; however, several astrovirus, including CAstV GA2011, GAstV HNNY0620, and GAstV TZ03, contain two s2m elements, based on Rfam analysis (Wang et al. 2021). Notably, three Malaysian chicken astrovirus isolates contain three s2m elements (Raji et al. 2021). The s2m element is highly conserved, and has been found in the genome of *Astroviridae*, *Picornaviridae*, *Caliciviridae*, and *Coronaviridae* (Tengs et al. 2013; Tengs and Jonassen 2016). The exact role of the s2m element remains obscure. Nonetheless, the s2m element was used as a target for antisense oligonucleotides in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) recently (Manfredonia et al. 2020; Lulla et al. 2021), indicating that the s2m element is a promising antiviral target. Future research might develop and assess anti-astrovirus agents targeting this element.

DAstV-5 possesses two NLS sequences. In contrast, the other avastroviruses possess only one NLS sequence. The NLS sequence mediates the process of viral protein entry into the nucleus, which influences virus replication and controls the host immune system (Chang and Hsia 2021). In Newcastle disease virus, the M protein enters into the nucleus via a bipartite NLS (Coleman and Peeples 1993). Mutation of the NLS mutation in the M protein of NDV resulted in attenuation of viral replication and pathogenicity in chicken fibroblasts and SPF chickens (Duan et al. 2018). When mutations take place in other viruses, such as Japanese encephalitis virus, H9N2 virus, and Venezuelan equine encephalitis virus, their replication and pathogenicity abilities changes (Mori et al. 2005; Xu et al. 2016; Lundberg et al. 2018). Therefore, we hypothesized that the two NLSs in DAstV-5 enhance the entry of virus proteins into the nucleus compared with the single NLS in other avastroviruses. However, the function of the two NLSs in DAstV-5 requires further study.

Although DAstV-3 CPH shares the lowest ORF2 amino acid genetic distance with the JM strain, the results demonstrated that these two strains are closely related to each other, but belong to different species. The JM strain shares genetic distances of 0.596 to 0.695 with the three official *Avastrovirus* species, indicating that JM could be classified as a novel species in the *Avastrovirus* genus. Furthermore, JM shares genetic distances of 0.402–0.662 with all the other known unassigned avastroviruses, revealing that it represents an additional unassigned avastrovirus. As with the other unassigned species, including DAstV-2, DAstV-3, DAstV-4, CAstV‑A, CAstV-B, GAstV, and NpAstV (Northern pintail astrovirus), the newly discovered strain JM is a new species of duck astrovirus. Therefore, we designated strain JM as DAstV-5.

Previous studies have revealed that some astroviruses are capable of transmitting across species (Donato and Vijaykrishna 2017; Smyth 2017; Roach and Langlois 2021). GAstV causes severe urate deposition in internal organs and joints, with mortality rates as high as 50% in geese in field case (Yang et al. 2018) and 75% in animal regression experiments (Niu et al. 2018). GAstV-induced duck gout began to prevalent in 2019. It causes symptoms of visceral and articular gout in duck, with a mortality rate of 10 to 30% in Cherry Valley duck and Muscovy duck in clinical cases (Wei et al. 2020; Chen, Zhang, et al. 2020; Chen et al. 2021). Moreover, GAstV can also cause clinical visceral gout in experimentally infected chickens (Li et al. 2021). The phenomenon that GAstVs cause gout in ducks and chickens demonstrates the cross-species transmission ability of goose astrovirus. However, the cross-species transmission ability of DAstV-5 is uncertain. The pathogenicity of DAstV-5 to chickens and geese should be further evaluated.

Recombination occurs in both human and non-human astroviruses (Wohlgemuth, Honce, and Schultz-Cherry 2019; Roach and Langlois 2021). In goose astroviruses, Chen *et al*. discovered a recombination event in ORF2 with duck astrovirus YP2 strain and TAstV-1 (Chen, Xu, et al. 2020). In another goose astrovirus, the JSHA strain, a potential recombination event was detected that related to the other two goose astroviruses, strains AHAU1 and SD01 (Wang et al. 2020). Meanwhile, recombination has taken place between human and non-human astroviruses, making them a likely zoonotic threat (Ulloa and Gutiérrez 2010; Karlsson et al. 2015; Hata et al. 2018). In the present study, we found no recombination events in DAstV-5 upon RDP analysis. However, we cannot rule out the possibility that DAstV‑5 might recombine with human astroviruses or other animal astroviruses in the future. Therefore, surveillance and identification of this new astrovirus are necessary.

In conclusion, we identified a novel duck astrovirus, the DAstV-5 JM strain, which induced visceral gout in ducklings. We used virome sequencing and analysis to obtain the complete genome sequence of the virus. Based on the phylogenetic analysis and pairwise comparisons, the DAstV-5 JM strain was identified as belonging to a novel species of avastrovirus. Our research lays the foundation to investigate the molecular epidemiology and etiology of this astrovirus causing duckling gout.

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TABLE 1. Five astrovirus segments identified by virome sequencing and analysis of DAstV-5 JM† strain

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Segments identified by sequencing | Length | Most similar sequence ID | Alignment similarity (%) | DNA or RNA |
| JMduck|contig\_420 | 1006 bp | KJ020899.1 | 73.60 | RNA |
| JMduck|contig\_708 | 685 bp | JF414802.1 | 70.20 | RNA |
| JMduck|contig\_817 | 661 bp | MN725025.1 | 61.60 | RNA |
| JMduck|contig\_1460 | 253 bp | KJ020899.1 | 77.00 | RNA |
| JMduck|contig\_1756 | 395 bp | EU143846.1 | 52.00 | RNA |

†Abbreviations: DAstV-5 JM strain, Duck Astrovirus Virus-5 JM strain

TABLE 2. Comparison of complete genome sequences and amino acid sequences of the three ORFs† of DAstV-5 JM strain with other representative strains of Avastroviruses

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Virus | GenBank accession no. | Sequence identity (%) | | | | Amino acid genetic distance  (ORF2) |
| **Genome**  **(nt)** | **ORF1a**  **(aa)** | **ORF1b**  **(aa)** | **ORF2**  **(aa)** |
| *Avastrovirus 1* | Turkey astrovirus 1 | Y15936 | 15 | 39 | 58 | 38 | 0.642 |
| *Avastrovirus 2* | Avian nephritis virus 1 | MN732559 | 20 | 26 | 55 | 29 | 0.695 |
| *Avastrovirus 3* | Turkey astrovirus 2 | NC\_005790 | 31 | 47 | 68 | 42 | 0.596 |
| Duck astrovirus C-NGB | NC\_012437 | 45 | 47 | 69 | 43 | 0.605 |
| Unassigned | Duck astrovirus SL1 | KF753804 | 41 | 49 | 69 | 41 | 0.605 |
|  | Duck astrovirus CPH | KJ020899 | 43 | 59 | 77 | 60 | 0.402 |
|  | Duck astrovirus YP2 | JX624774 | 41 | 41 | 64 | 48 | 0.524 |
|  | Goose astrovirus SDXT | MN399857 | 29 | 48 | 68 | 36 | 0.662 |
|  | Goose astrovirus FLX | KY271027 | 21 | 48 | 65 | 37 | 0.641 |
|  | Goose astrovirus HN1G | KY807085 | 34 | 48 | 68 | 36 | 0.658 |
|  | Chicken astrovirus Poland/G059/2014 | KT886453 | 26 | 54 | 79 | 43 | 0.586 |
|  | Chicken astrovirus GA2011 | JF414802 | 34 | 53 | 79 | 55 | 0.440 |
|  | MPJ1433/Northern pintail/091230 | JX985651 | - | - | - | 24 | 0.777 |

†Abbreviations: DAstV-5 JM strain, Duck Astrovirus Virus-5 JM strain; ORF, open reading frame



FIGURE 1. Pathological lesions of clinical samples. (A) Urate deposition in the heart; (B) Urate deposition in the mesenterium; (C) Urate deposition in the gallbladder.

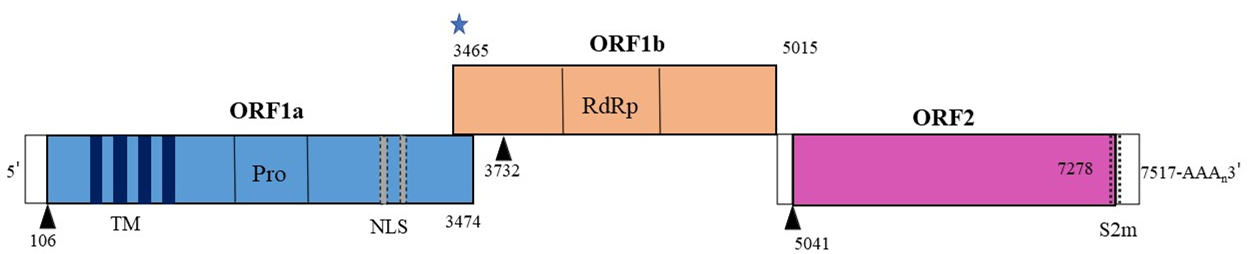


FIGURE 2. Genome organization of DAstV-5 JM strain. The three predicted ORFs and the typical genomic characteristics are shown. The black triangles represent the translation start site of the three ORFs. The blue star represents the heptameric AAAAAAC sequence. DAstV-5 JM strain, Duck Astrovirus Virus-5 JM strain; ORF, open reading frame.

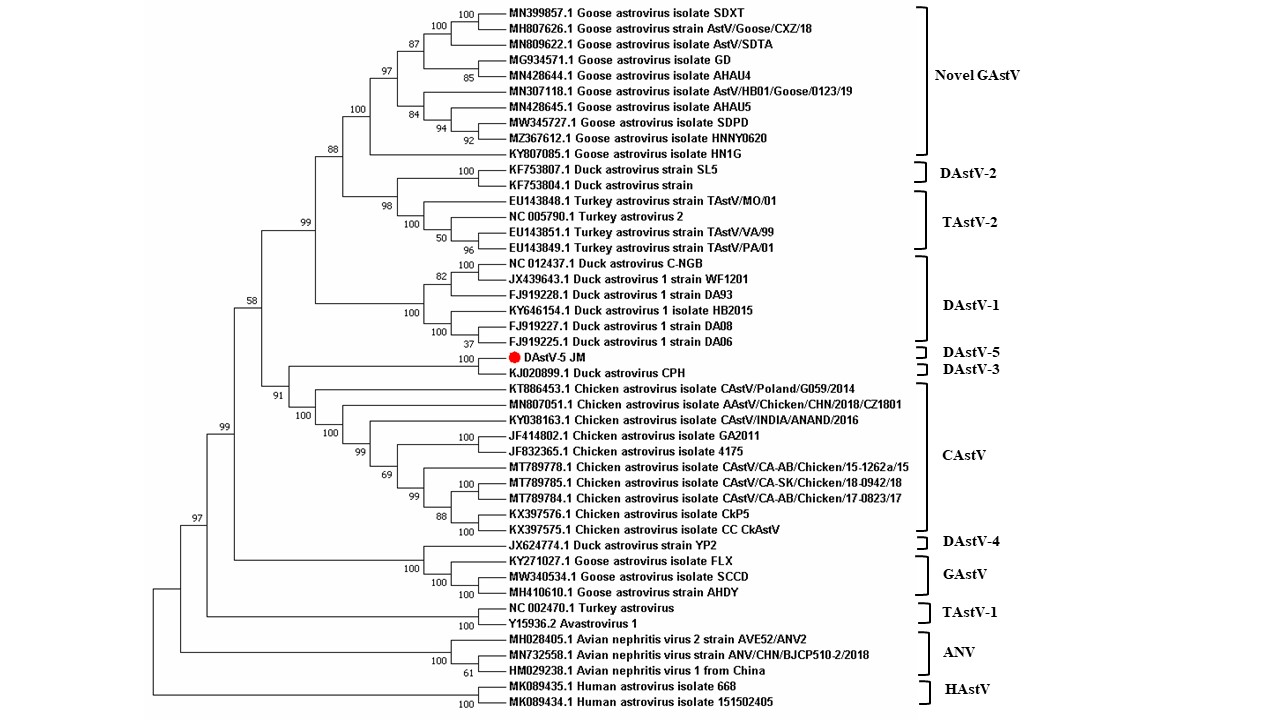
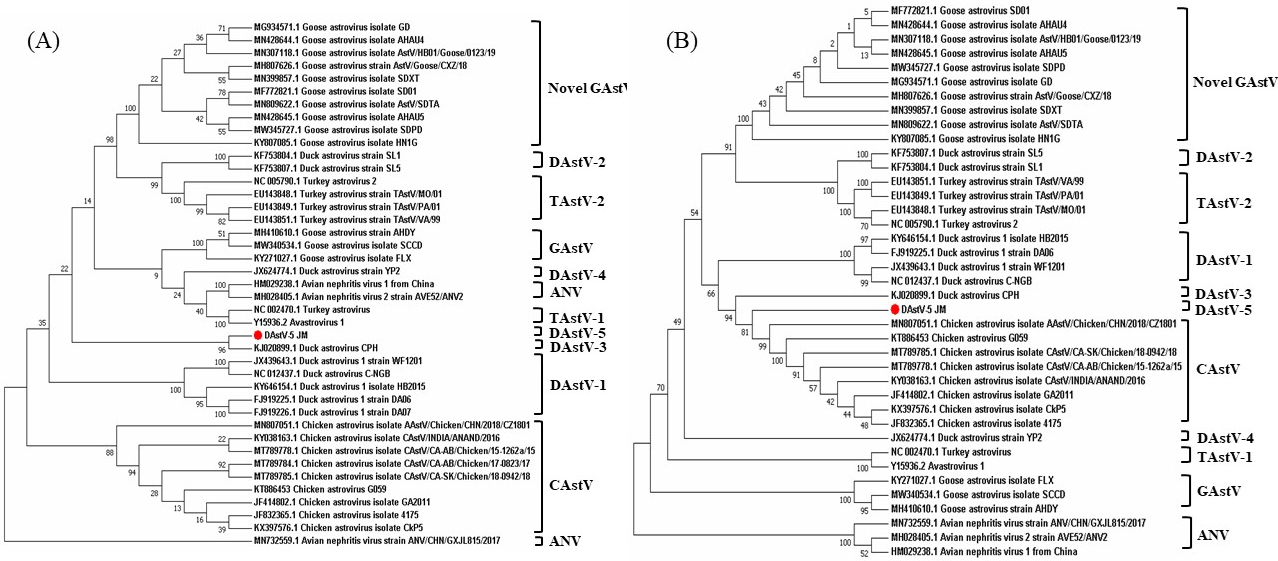


FIGURE 3. Phylogenetic analysis of the complete genome sequences of DAstV-5 JM strain with other astroviruses. The phylogenetic tree was constructed using MEGA 7.0 software using the neighbor-joining method, with 1000 bootstrap replications. DAstV-5 JM strain, Duck Astrovirus Virus-5 JM strain.



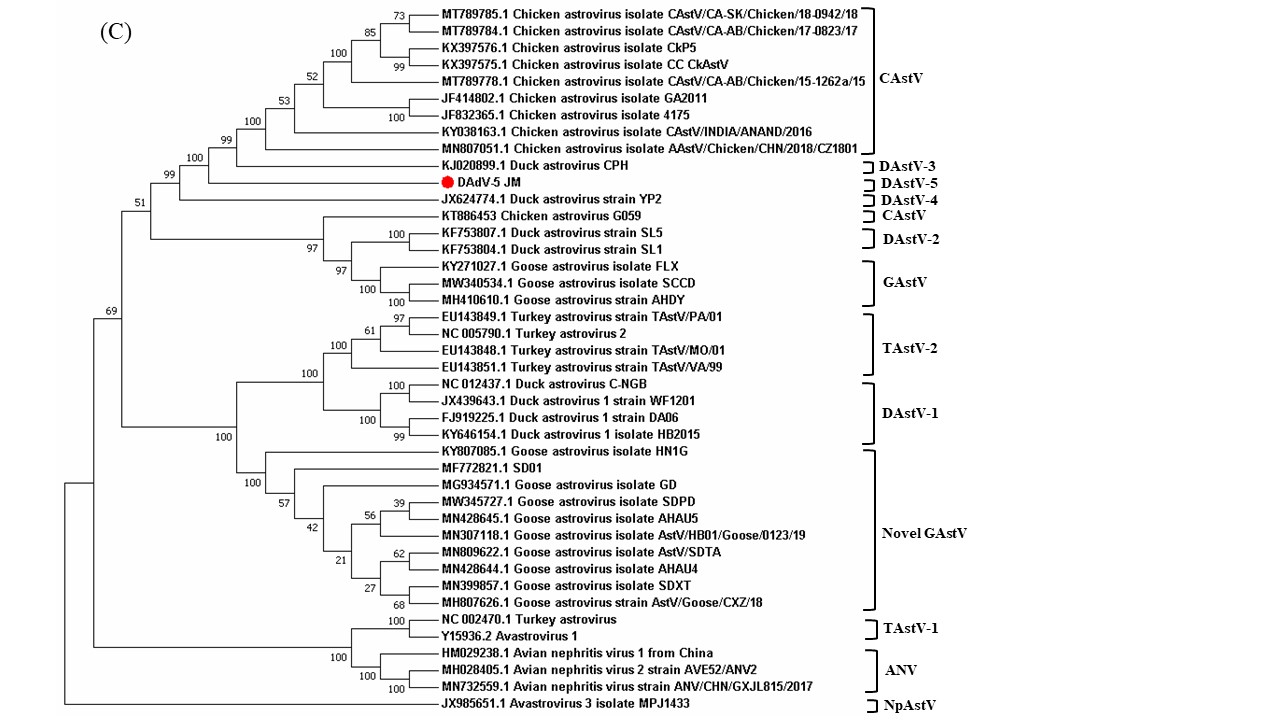


FIGURE 4. Phylogenetic analysis of the amino acid sequences of ORF1a (A), ORF1b (B), and ORF2 (C) of the DAstV-5 JM strain and other astroviruses. The phylogenetic trees were constructed using MEGA 7.0 software using the neighbor-joining method, with 1000 bootstrap replications. DAstV-5 JM strain, Duck Astrovirus Virus-5 JM strain; ORF, open reading frame.