Isolation and characterization of a goose astrovirus 1 strain causing fatal gout in goslings, China

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ABSTRACT In recent years, goose gout, a severe infectious disease, has affected the development of the goose industry in China. Two different genotypes of goose astrovirus (**GAstV**), named as GAstV-1 and GAstV-2, were identified. GAstV-2 viruses are known to be the causative agent of goose gout; however, GAstV-1 has not been isolated, and the relationship between GAstV-1 and goose gout is unknown. One full genome sequence, designated as GAstV/CHN/TZ03/2019 (TZ03), was determined from the clinical tissue samples of a diseased gosling using next-generation sequencing. The complete genome of TZ03 was 7,262 nucleotides in length with typical genomic characteristics of avastroviruses. The TZ03 strain shares the highest identity (96.6%) with the GAstV-1 strain FLX, but only 51.5 to 61.3% identity with other astroviruses in *Avastrovirus*. Phylogenetic analysis revealed that the TZ03 strain clustered together with the GAstV-1 strains FLX and AHDY and was highly divergent from GAstV-2 viruses. The TZ03 strain was successfully isolated from goose embryos and caused 100% mortality of goose embryos after 5 passages. Electron microscopy showed that the virus particles were spherical with a diameter of ∼22 nm. The clinical symptoms were reproduced by experimental infection of healthy goslings, which were similar to those caused by GAstV-2 strains. Our data show that GAstV-1 is one of the causative agents of the ongoing goose gout disease in China. These findings enrich our understanding of the evolution of GAstVs that cause gout and provide potential options for developing biological products to treat goose gout.

Key words: astrovirus, causative agent, goose gout, next-generation sequencing

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INTRODUCTION

Since 2014, there has been an outbreak of an infectious disease that mainly causes gout symptoms, characterized by diarrhea, lameness, or paralysis, in 4- to 20-day-old goslings in China (Zhang et al., 2017; Yang et al., 2018). Autopsies have showed that the affected goslings exhibit numerous uric acid deposits in the visceral organs and joints (Wu et al., 2020). The mortality rate is as high as 50% (Yang et al., 2018), resulting in serious economic losses to the goose industry in China. Although various factors can cause gout in geese, including high-protein feed, vitamin A or vitamin D deficiency, drug misuse, and mismanagement (Chen et al., 2019), an increasing number of studies have indicated that goose astroviruses may be the causative pathogens (**GAstVs**) (Liu et al., 2018; Wan et al., 2018; Zhang et al., 2018b).

Astroviruses are nonenveloped, positive-sense, single-stranded RNA viruses first identified in 1975 in the fecal samples of children with gastroenteritis (Madeley and Cosgrove, 1975). The family *Astroviridae* comprises 2 genera, *Mamastrovirus* and *Avastrovirus*, comprising astroviruses that infect mammalian and avian species, respectively (Chu et al., 2008). Of the avian species, astroviruses have been reported in turkeys (**TAstV**) (Jonassen et al., 2003), ducks (**DAstV**) (Liu et al., 2014), chickens (**CAstV**) (Todd et al., 2009), guinea fowls (**GFAstV**) (Cattoli et al., 2007), pigeons (**PiAstV**) (Zhao et al., 2011), geese (Niu et al., 2018; Chen et al., 2019), and wild aquatic and terrestrial birds (Chu et al., 2012; Donato and Vijaykrishna, 2017). To date, the International Committee on Taxonomy of Viruses has recognized three species (*Avastrovirus* 1, *Avastrovirus* 2, and *Avastrovirus* 3) in the genus

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Table 1. Primers used for virus detection.

Primer name	Sequence (5'-3')	Target	Reference
GPV-F	AGACTTATCAACAACCATCAT(C) T	VP1 gene of GPV (779 bp)	(Todd et al., 2009)
GPV-R	TCACTTATTCCTGCTGTAG		
GHPV-F	GAGGTTGTTGGAGTGACCACAATG	VP1 gene of GHPV (144 bp)	(Guerin et al., 2000)
GHPV-R	ACAACCCTGCAATTCCAAGGGTTC		
REOV-F	GGTGCGACTGCTGTATTTGGTAAC	S1 gene of REOV (513 bp)	(Niu et al., 2017)
REOV-R	AATGGAACGATAGCGTGTGGG		
TMUV-F	GCCACGGAATTAGCGGTTGT	E gene of TMUV (401 bp)	(Su et al., 2011)
TMUV-R	TAATCCTCCATCTCAGCGGTGTAG		
FAdV-F	CAACTACATCGGGTTCAGGGATAACTTC	Hexon gene of FAdV (766 bp)	(Ye et al., 2016)
FAdV-R	CCAGTTTCTGTGGTGGTTGAAGGGGTT		
GAstV-F	CGGTGGAATACATCAGCGAGTA	RdRP gene of GAstV (390 bp)	(Yuan et al., 2019)
GAstV-R	CCTTCCTTATTGACACAAGCCTAT		

Avastrovirus, among which *Avastrovirus* 1 includes turkey astrovirus 1 (**TAstV-1**), *Avastrovirus* 2 includes 2 types of Avian nephritis virus (**ANV-1** and **ANV-2**), and *Avastrovirus* 3 includes duck astrovirus 1 (**DAstV-1**) and turkey astrovirus 2 (**TAstV-2**). Many *Avastroviruses* still require classification, particularly those isolated from waterfowl (ducks and geese).

The whole genome sequence of the GAstV FLX was first reported in 2017 (Zhang et al., 2017). This genome is 7,299 nucleotides (**nt**) in length, consists of 3 overlapping open reading frames (**ORFs**) in different reading frames, and shows genomic characteristics similar to those of other reported astroviruses. Several GAstV strains, such as SD01, GD, CXZ/18, SDPY, JSHA, and HN1G, have been reported and sequenced (Liu et al., 2018; Niu et al., 2018; Wan et al., 2018; Yang et al., 2018; Zhang et al., 2018b; Chen et al., 2019). All sequenced GAstVs belonged to 2 distinct phylogenetic clades (Zhang et al., 2018b). Therefore, the FLX-like strain is proposed as GAstV-1 and the SD01-like strain is proposed as GAstV-2 (Wan et al., 2018). Although it is difficult to isolate GAstVs, several GAstV-2 strains such as SD01, SDPY, GD, and CXZ18 have been isolated successfully, and the relationship between the viral pathogenicity and goose gout was determined (Yang et al., 2018; Zhang et al., 2018a). For GAstV-1, only 2 strains, FLX and AHDY, were reported and sequenced, but these were not isolated and their pathogenicity has not been verified (Zhang et al., 2017). In this study, we isolated and characterized a GAstV-1 strain and evaluated its pathogenicity by experimental infection of goslings.

MATERIALS AND METHODS

Ethics Statement

The animal experiments were approved by the Ethical Committee for Animal Experiments of Jiangsu Agri-animal Husbandry Vocational College and conducted in accordance with the Guidelines for Experimental Animals of the Ministry of Science and Technology (Beijing, China).

Sample Origin and Processing

In August 2019, a disease outbreak occurred in a commercial *Yangzhou* geese farm (about 10,000 goslings) without vaccination history located in Taizhou, Jiangsu Province, China. The outbreak resulted in morbidity of approximately 40% in 1- to 3-wk-old goslings. Liver, heart, and kidney tissues were collected from dead goslings with typical symptoms of gout. Tissues from the same bird were pooled and homogenized for further analysis.

Bacteria Isolation and PCR for Virus Detection

For bacteriological diagnosis, the homogenates were first inoculated onto tryptic soy agar plates (BD Biosciences, Franklin Lakes, NJ) containing 2% fetal calf serum and incubated at 37°C under an atmosphere of 5% CO_2 for 48 h. Homogenates were tested for the presence of goose parvovirus (**GPV**), goose hemorrhagic polyomavirus (**GHPV**), reovirus (**REOV**), Tembusu virus (**TMUV**), fowl adenovirus (**FAdV**), and GAstV-2. The primers used for virus detection are listed in Table 1.

Next-Generation Sequencing

To detect possible pathogens, we performed metagenomic sequencing using the pooled tissue samples as previously described (Zhang et al., 2016). Specifically, DNA in the extracted DNA/RNA from these samples was removed using an RNase-Free DNase Set (Qiagen, Hilden, Germany), and the reagent residual was removed from the remaining RNA using an Agencourt RNA Clean XP kit (Beckman Coulter, Brea, CA,) according to the manufacturer's instructions. The library was prepared using a NEXT flex Rapid RNA-Seq Kit (Bioo Scientific, Austin, TX) until "Step D" according to the kit's manual (with minor modification), followed by using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA). The normalized library was sequenced on the MiSeq platform (Illumina) with a 300-cycle MiSeq Reagent Micro Kit V2 (Illumina).

Raw sequencing data were cleaned by removing adapters, trimming low-quality ends, depleting sequences with lengths of less than 36 nt, and analyzing sequencing quality using FastQC (Bolger et al., 2014). Cleaned reads were taxonomically classified using Kraken v0.10.5-beta (Wood and Salzberg, 2014). Reads of the viruses of interest were extracted from the Kraken classification results and de novo assembled with SPAdes (v 3.5.0) (Robinson et al., 2011).

Sequence Analysis

Transmembrane (**TM**) domains in ORF1a were predicted using TMHMM (http://www.cbs.dtu.dk/services/TMHMM), and functional regions in the proteins were predicted using Pfam (http://pfam.xfam.org). The stem ring structures of nucleic acids were analyzed using Rfam (http://rfam.xfam.org). Referenced sequences of other avian AstVs were downloaded from the GenBank database. Multiplex sequence alignments were generated using Muscle. Phylogenetic trees were constructed from aligned sequences using the neighbor-joining method with the maximum composite likelihood model in MEGA version 7 as previously reported (Wan et al., 2018). The robustness of the phylogenetic tree was evaluated by bootstrapping using 1,000 replicates. The sequence alignment was subjected to detection of homologous recombination using Recombination Detection Program 4 (Martin et al., 2015).

Virus Isolation

To isolate GAstV-1, the homogenates were centrifuged at 8,000 × *g* at 4°C for 30 min, and the supernatants were filtered through a 0.2-μm syringe-driven filter. The filtered suspension was then inoculated into the chorioallantoic cavity of 9-day-old specific pathogen-free (**SPF**) chicken embryos (Lihua Agricultural Technology Co., Zhejiang, China), 10-day-old SPF duck embryos (Shandong Academy of Agricultural Sciences, China), and 11-day-old goose embryos (Jinpeng Co., Jiangsu, China). The goose embryos that tested negative by PCR using GAstV-2-specific primers and GAstV-1-specific primers (GAstV1-F: 5'-CTGGTTTCGCTGAGACTCCT-3', GAstV1-R: 5'-GGCCCAACTTCTGGTAGCTT-3') were utilized for virus isolation. The embryos were incubated at 37°C and monitored daily. Embryos that died after 24 h or survived until 7 d after inoculation were chilled to 4°C overnight. The allantoic fluids were collected for the detection of GAstV-1 and further passaging. In parallel, total RNA was extracted from the allantoic fluids and identified by reverse-transcription (**RT**)-PCR with GAstV-1-specific primers.

Purification and Electron Microscopy of Virus Isolates

To identify the isolates morphologically, the viral particles were purified and identified by electron microscopy (**EM**). The allantoic fluid collected during the virus isolation step was first centrifuged at 8,000 × *g* at 4°C for 30 min, and the resulting supernatant was further ultracentrifuged at 100,000 × *g* at 4°C for 2 h (Hitachi CS120GXⅡ, S50A rotor, Tokyo, Japan). The resulting pellet was resuspended in PBS and loaded on top of preformed 10 to 60% sucrose gradients. The sucrose gradients were centrifuged at 100,000 × *g* at 4°C for 1 h (Hitachi CS120GXⅡ, S52ST rotor, Tokyo, Japan), after which the gradients were fractionated. The collected fractions were strained for 1 min with 2% phosphotungstic acid (pH 7.4) and observed under a transmission electron microscope (HT7800, Japan).

Experimental Replication of the Disease

To validate the pathogenicity of the isolated GAstV-1, artificial infection experiments were conducted in 24 three-day-old healthy goslings (Jinpeng Co.). The goslings were randomly divided into 2 groups (A and B, n = 12/group) and reared in separate isolators. Goslings in group A were inoculated by subcutaneous injection with 0.3 mL of the isolated virus ($10^{3.25}$ ELD_{50}/0.3 mL), and goslings in group B were inoculated by subcutaneous injection with 0.3 mL of sterile PBS (pH 7.2) as a negative control. Goslings were monitored daily for mortality, and tissues of dead goslings were collected for pathological examination, virus isolation, and identification.

RESULTS

Case History and Microbiological Examination of Samples

In 2019, geese at a goose farm in Jiangsu province experienced gout with a mortality rate of approximately 40%. The goslings showed typical symptoms of gout, including depression, paralysis, and leg joint enlargement. At necropsy, uric deposits in the liver, heart, and articular cavity, as well as severe hemorrhage and kidney swelling were observed in the dead goslings. Mild meningeal hemorrhage was observed in some cases (Figure 1). The disease lasted for approximately 10 d, and surviving goslings grew slowly. However, no virulent bacteria were isolated, and tissue samples tested negative for GPV, GHPV, REOV, TMUV, FAdV, and GAstV-2 according to PCR.

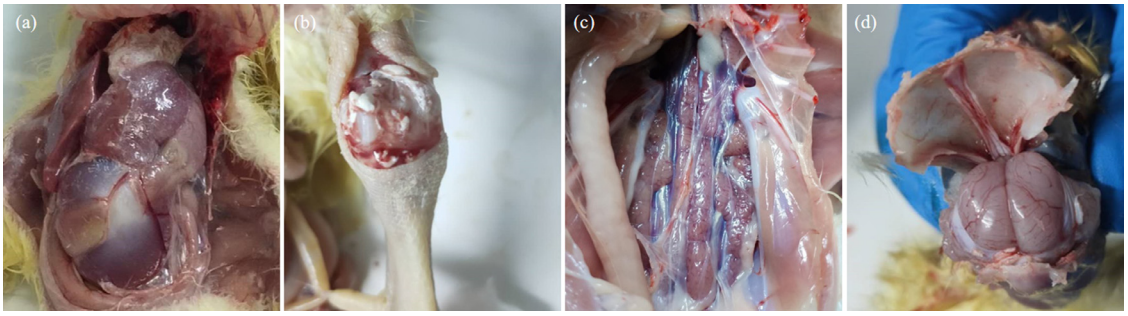


Figure 1. Pathological lesions of clinical samples. (A) Uric deposits on the surface of the liver and heart. (B) Uric deposits in the articular cavity. (C) Severe hemorrhage and swelling of the kidneys. (D) Mild hemorrhage on the meninges.

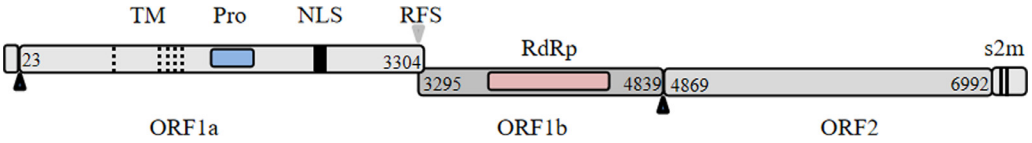


Figure 2. Predicted genome organization of GAstV TZ03.The nucleotide position of the three predicated ORFs (including stop codons) in the genome is shown. The translation start sites of ORF1a and ORF2 are indicated by black triangles, and the start site of the ribosomal frameshift signal sequence AAAAAAC is shown by gray triangles. Abbreviations: NLS, nuclear localization signal; ORF, open reading frame; Pro, protease; RFS, ribosomal frameshift signal; RdRp, RNA-dependent RNA polymerase; s2m, stem-loop II-like motif; TM, transmembrane domain.

Genome Sequence Analysis

Because no virulent bacteria or viruses were detected using the traditional method, we performed metagenomic NGS on the Illumina MiSeq platform to detect potential pathogens. After using an in-house bioinformatics analysis pipeline, one long contig identified as an astrovirus was generated and deposited in GenBank under accession number MW353015. No other viral sequences were detected after quality control and analysis.

The complete genome, designated as GAstV/CHN/TZ03/2019 (TZ03), was 7,262 nt in length with the typical genomic characterization of other known avastroviruses (Figure 2), consisting of a 5'-untranslated region (UTR) of 22 nt, 3 overlapping ORFs (ORF1a, 3,282 nt; ORF1b, 1,545 nt; ORF2, 2,124 nt), and a 3'-UTR of 270 nt.

The three ORFs were in different reading frames, as observed in other astroviruses. A ribosomal frameshift signal (AAAAAAC) and downstream stem-loop structure (3,308−3,332 nt) were identified in ORF1a. The conserved CCGAA motif of astroviruses was found at the 5' end of the genome, as well as the space between the ORF1b stop codon and ORF2 start codon. Two stem-loop-II-like (**s2m**) motifs (7,004−7,046; 7,099−7,141 nt) were found in the 3'-UTR based on Rfam analysis, which somewhat differs from other known goose astroviruses. ORF1a and ORF1b of TZ03 contained the characteristic motifs conserved in other astroviruses (Arias and DuBois, 2017), including a serine protease motif (685GNSG688), an NLS (residues 784−815), and an RdRp (residues 175−411). Five TM domains were identified at positions 217−239, 371−388, 401−423,433−455, and 468−490 of ORF1a.

The amino acid (**aa**) sequences of the three ORFs and whole-genome sequence of the TZ03 strain were compared with representative members of the genus *Avastrovirus*; the results are summarized in Table 2. The TZ03 strain shares the highest identity with the goose astrovirus strain FLX in terms of the whole-genome sequence (96.6%) and aa sequence (ORF1a: 99.5%, ORF1b: 98.7%, ORF2: 97.2%). The TZ03 strain displayed relatively low identity with other avastroviruses in terms of the whole-genome sequence (51.5−61.3%) and aa sequence (ORF1a: 35.4−54.6%, ORF1b: 53.8−66.3%, ORF2: 31.2−48.5%), including the SD01 strain also isolated from geese.

Phylogenetic and Recombination Analysis

To investigate the evolutionary relationships between the TZ03 strain and other members of the genus *Avastrovirus*, phylogenetic trees were constructed (Figure 3). The phylogenetic tree of whole-genome sequences and amino acid sequences of ORF1a, ORF1b, and ORF2 all indicated that the TZ03 strain clustered together with GAstV FLX and AHDY in clade GAstV-1 and was highly divergent from the other reported GAstVs in clade GAstV-2. Trees based on ORF1a exhibited similar topology patterns for GAstV TZ03 as those derived from whole-genome sequence analysis, and GAstV-1 strains grouped with DAstV-1, DAstV-3, and CAstV. Although the phylogenetic tree based on ORF2 revealed a distinctive topology pattern for GAstV TZ03, the virus was more closely related to DAstV-2 strains than to other avastroviruses. Recombinant analysis was conducted based on the alignment of all available genomes of avastroviruses, with the results revealing no obvious recombination event.

Table 2. Percentage identity between genomic sequences, amino acids sequences of the three ORFs of GAstV TZ03, and representative members of the genus *Avastrovirus*.

				Sequence identity (%)			
Avastrovirus species	Virus	Strain	GenBank accession no.	Genome	ORF1a	ORF1b	ORF2
Avastrovirus 1	TAstV-1	-	NC002470	54.8	44.9	55.7	43.0
Avastrovirus 2	ANV	BJCP510-2	MN732558	51.5	35.4	53.8	31.2
Avastrovirus 3	TAstV-2	CO/01	EU143845	58.7	53.5	64.0	41.0
	DAstV-1	C-NGB	FJ434664	57.7	50.8	64.4	40.9
Unassigned	DAstV-2	SL4	KF753806	61.3	54.4	66.3	52.6
	DAstV-3	CPH	KJ020899	57.5	51.6	63.6	38.4
	DAstV-4	YP2	JX624774	56.7	49.6	59.8	40.9
	CAstV-A	G059	KT886453	60.1	54.6	66.1	48.5
	CAstV-B	GA2011	JF414802	58.5	54.2	66.1	40.6
	GAstV	SD01	MF772821	58.2	54.0	60.5	43.1
	GAstV	FLX	KY271027	96.6	99.5	98.7	97.2
	GAstV	AHDY	MH410610	92.1	99.5	99.6	82.9

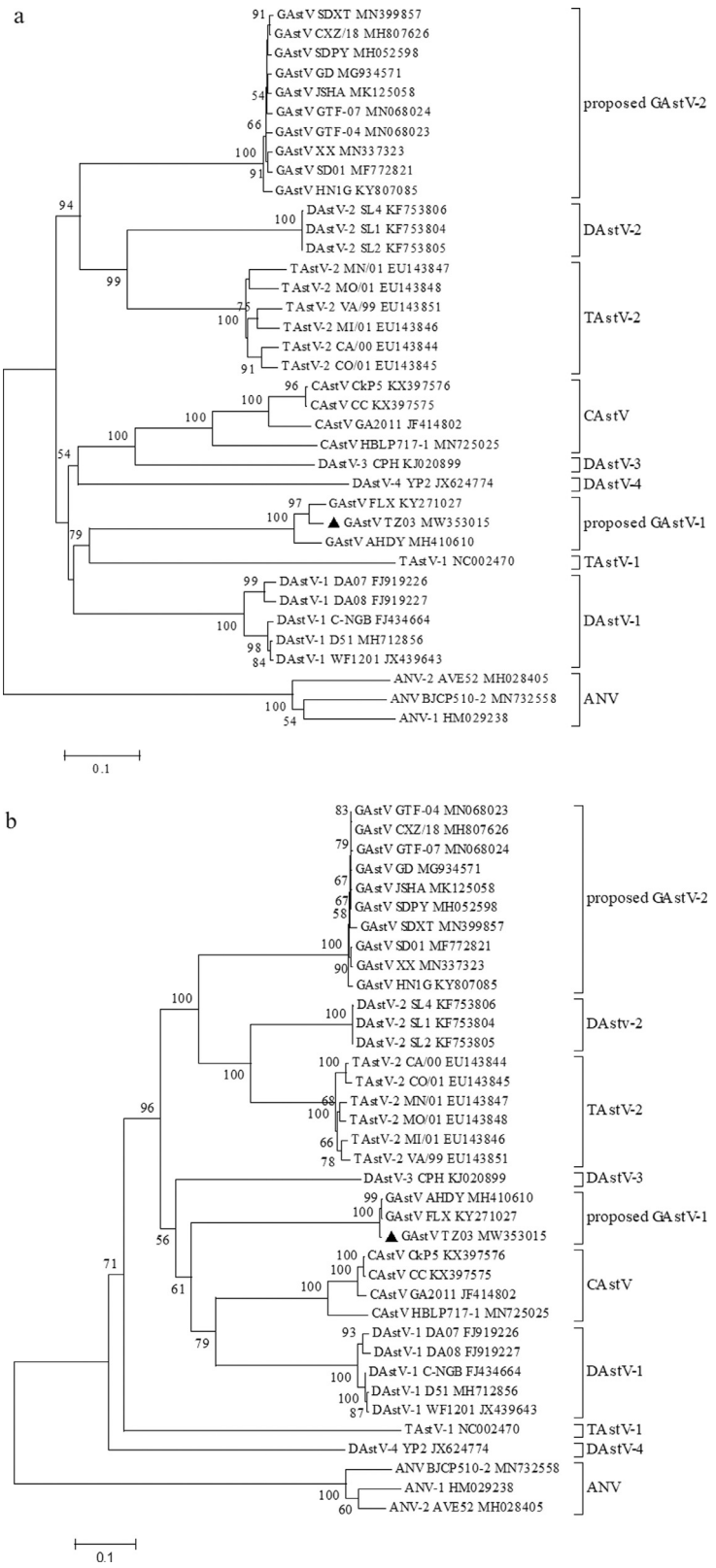
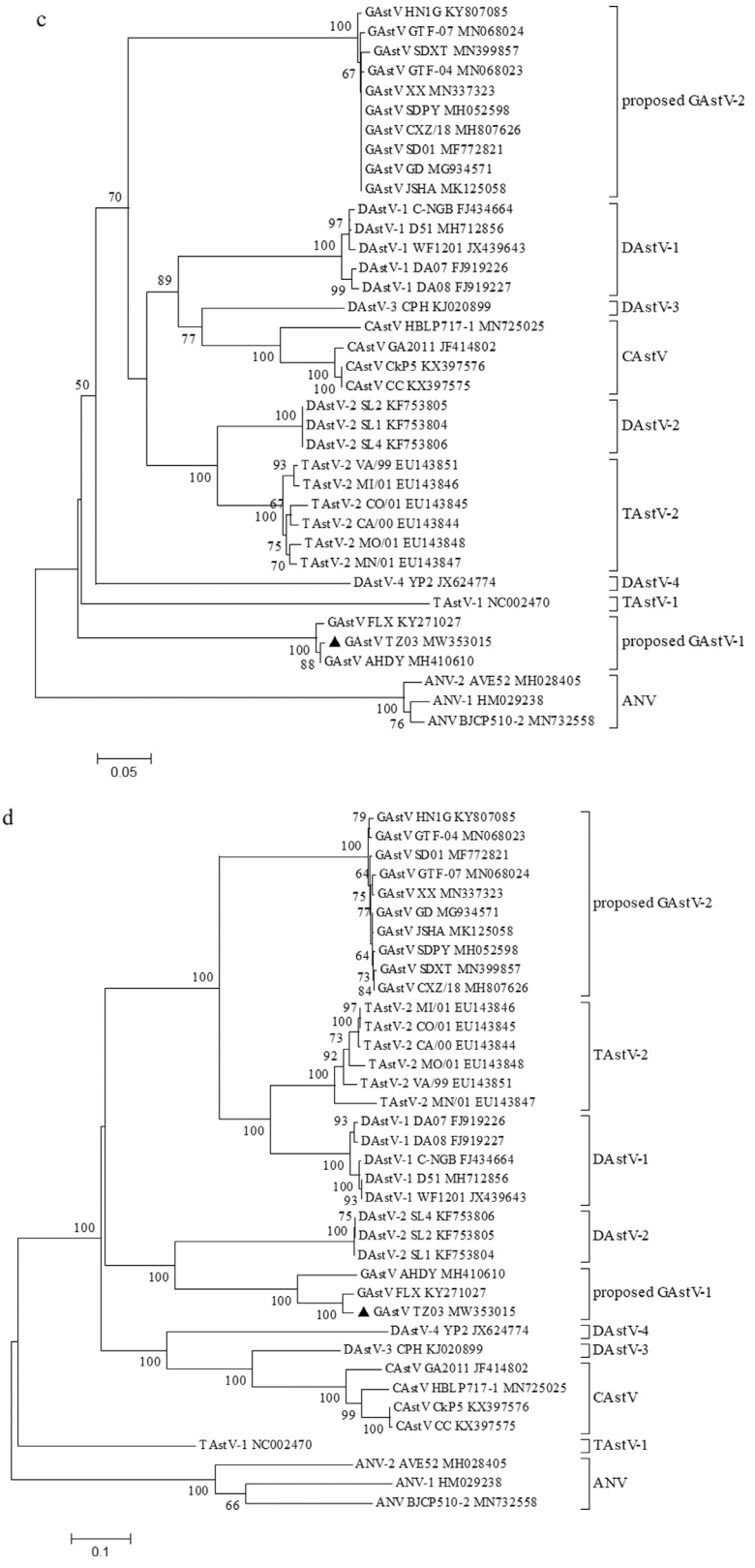


Figure 3. Phylogenetic analysis of goose astrovirus. The phylogenetic trees based on whole genome sequences (A) and amino acid sequences of ORF1a (B), ORF1b (C), and ORF2 (D) were constructed using the neighbor-joining method with 1,000 bootstrap replicates and the maximum composite likelihood model. The GAstV TZ03 isolate determined in this work is marked with a black triangle.



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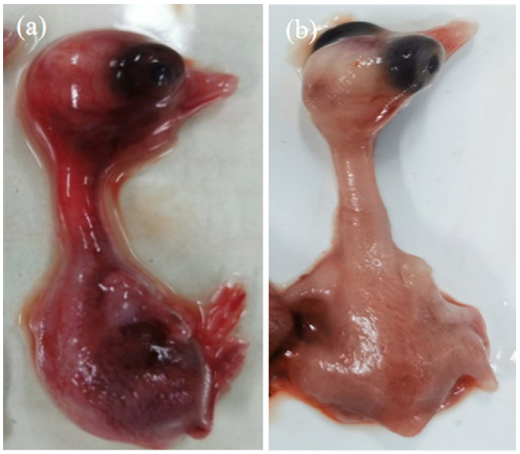

Figure 4. Pathological changes in goose embryos infected with goose astrovirus. (A) Infected goose embryos at 48−72 h postinfection, showing hemorrhage; (B) noninfected goose embryo control.

Isolation of GAstV-1 in Goose Embryos

To isolate the TZ03 strain, the homogenates were inoculated into SPF chicken, SPF duck, and goose embryos. In goose embryos, no deaths had occurred by 10 d postinoculation within 3 passages, but the subsequent passage caused 40 to 100% mortality of the goose embryos by 10 d postinoculation. Dead embryos exhibited severe subcutaneous hemorrhage (Figure 4), and allantoic fluids were positive for GAstV-1 by gene-specific RT-PCR. No deaths or pathological changes were found in chicken or duck embryos within 5 passages, and the results of RT-PCR were negative. The purified virus particles were spherical and approximately 22 nm in size, as revealed by electron microscopy (Figure 5).

Experimental Replication of the Disease

After infection with the TZ03 strain, 5 of the 12 infected goslings died within 6 to 8 d, resulting in a mortality rate of 41.7%. At necropsy, symptoms of hemorrhage and swelling of the kidneys and uric deposition in the heart, liver, and leg joints were noticeable (Figures 6A−6C), and meningeal hemorrhage was observed in some goslings (Figure 6D). These symptoms are similar to those found in clinical cases. Histological examination revealed red staining protein-like substances in the renal tubules (Figure 6G); necrosis of renal tubular epithelial cells and hepatocytes; and inflammatory cell infiltration in the heart, kidney, and liver (Figures 6E−6G). Cerebral vascular congestion and glial cell proliferation was observed in the brain (Figure 6H). Following embryo inoculation, TZ03 was successfully reisolated from infected/dead goslings and its presence was confirmed by RT-PCR (Figure 7) and sequencing.

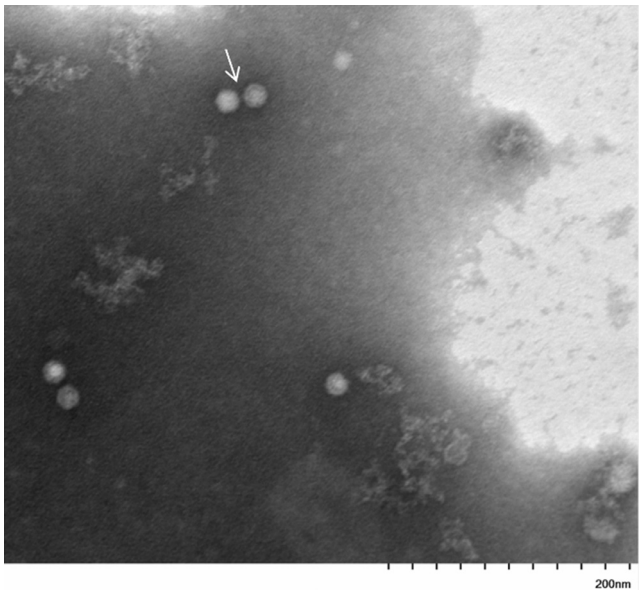

Figure 5. Electron microscopic analysis of purified GAstV-1 TZ03 stained with 2% phosphotumgstic acid. Original magnification: × 70,000. Bar: 200 nm.

DISCUSSION

Since 2014, persistent goose gout has caused great economic losses in the goose breeding industry. Several researchers have shown that the pathogen associated with the disease is a goose astrovirus. In the present study, the GAstV-1 strain TZ03 was first isolated from dead goslings, its complete genome was determined by NGS, and its pathogenicity was determined in replication experiments.

According to previous reports, GAstV-2 is the most prevalent strain causing goose gout in China (Yuan et al., 2019). A pair of GAstV-2-specific primers was used to detect clinical samples with typical gout symptoms, but the result was negative; the samples also tested negative for other pathogens. As a powerful tool for detecting pathogens, NGS technology was used. After quality control and alignment, a complete genome sequence of the TZ03 strain with a total size of 7,262 nt was obtained; no other viral sequences were detected in analysis of the obtained sequences. The results suggest that TZ03 was the only pathogen in the clinical sample.

The whole genome of TZ03 shared 96.6% nucleotide identity with the GAstV-1 strain FLX, whereas only 58.2% with the GAstV-2 strain SD01. Phylogenetically, TZ03 clustered closely with the GAstV-1 strain FLX and AHDY, but distantly from the clade formed by other GAstV strains. These data indicate that TZ03 belongs to GAstV-1. The TZ03 genome possessed the typical genomic characteristics and conserved motifs of astroviruses, including a ribosomal frameshift signal, a serine protease motif, and an NLS. Five TM domains are present in the ORF1a protein as reported in FLX, whereas only 4 TM domains are present in the ORF1a protein of GAstV-2 strains (Liu et al., 2018; Yang et al., 2018; Zhang et al., 2018a; Chen et al., 2019). Moreover, two s2m motifs exist in the 3'-UTR, whereas only one s2m motif exists in other goose astroviruses (Zhang et al., 2017; Liu et al., 2018). Whether these structural differences affect the replication and pathogenicity of the virus requires further exploration.

It is well known that astroviruses are difficult to isolate. To date, only a small number of GAstV-2 strains

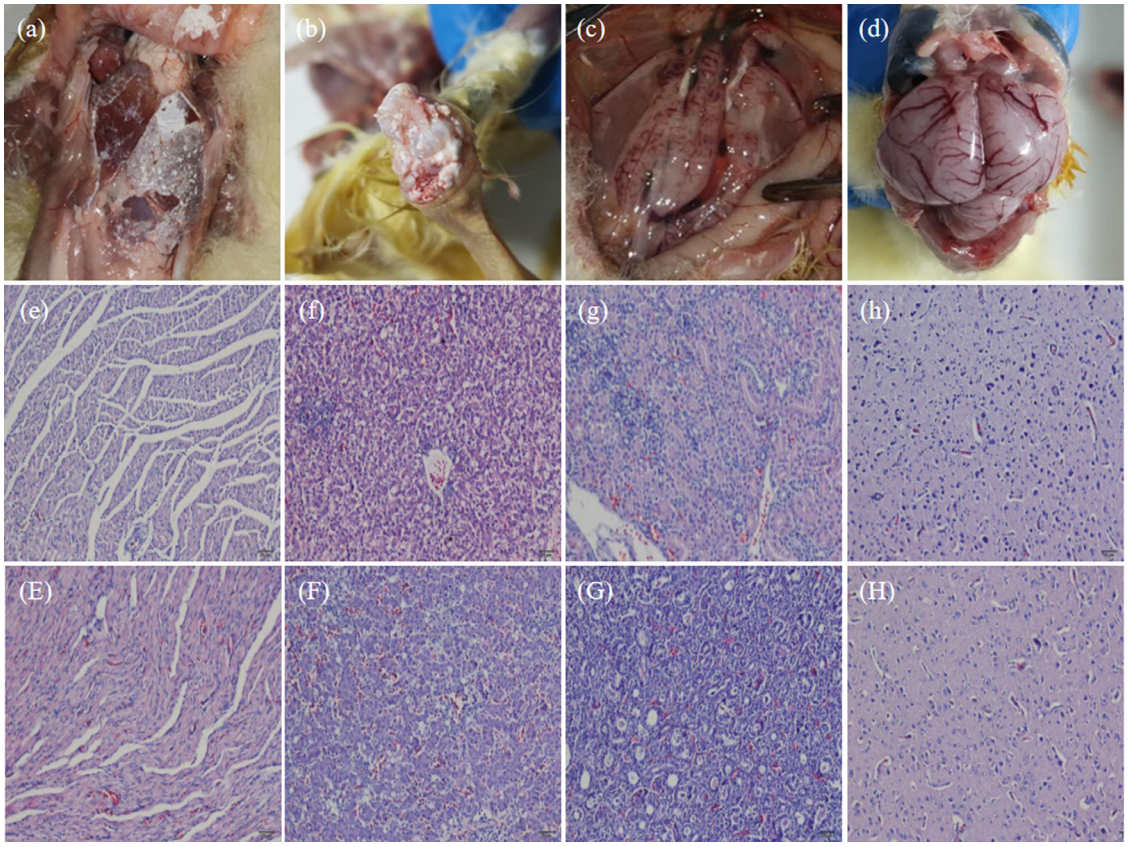

Figure 6. Pathological and histopathological lesions in geese experimentally infected with the TZ03 strain. (A) Uric deposits in visceral organs. (B) Uric deposits in the articular cavity. (C) Severe hemorrhage and swelling of kidneys and uric deposits in ureters. (D) Hemorrhage on the meninges. (E) Hematoxylin and eosin (HE)-stained heart section with myocardial fiber swelling and rupture, inflammatory cell infiltration. (F) HE-stained liver section, hepatocyte necrosis and heterophilic granulocyte infiltration. (G) HE-stained kidney section, red staining protein like substances in the renal tubules and inflammatory cell infiltration. (H) HE-stained brain section, cerebral vascular congestion, and glial cell proliferation. (E−H) Negative control (histology changes in healthy geese).

have been successfully isolated. The strains SD01 and CXZ18 were isolated from goose embryos (Zhang et al., 2018a; Yuan et al., 2019).The strains GD and SDPY were isolated in the chicken liver cell line LMH (Zhang et al., 2018b) and SPF chicken embryos (Yang et al., 2018), respectively. Although the complete sequences of 2 GAstV-1 strains, FLX and AHDY, were reported, none were isolated successfully. Goose, SPF chicken, and SPF duck embryos were used to isolate the GAstV-1 strain TZ03. No goose embryos died during their first 3 passages. After 5 passages, the mortality rate reached 100%. Furthermore, no deaths or pathological changes were found in SPF chicken or duck embryos even after 5 passages, and the RT-PCR results were negative. These data show that neither chicken nor duck embryos are suitable for GAstV-1 virus isolation. Human astrovirus can be cultured in Caco-2 cells in the presence of trypsin, and viral replication ability increases with increasing trypsin concentrations (Méndez et al., 2002; Arias and DuBois, 2017). We also attempted to isolate TZ03 in LMH but failed, even in the presence of trypsin (data not shown).This result is inconsistent with a previous study showing that GAstV-2 strain GD can be cultured in LMH in the presence of trypsin (Zhang et al., 2018b). The replication characteristics of GAstV-1 may differ from those of GAstV-2. Whether the GAstV-1 TZ03 isolate can replicate in other cell lines and if trypsin can promote the replication of GAstV-1 should be further studied. The diameter of the purified GAstV-1 TZ03 virus particles was approximately 22 nm, which is smaller than that of the mammalian astrovirus (approximately 41 nm) (Arias and DuBois, 2017). This may be attributed to the fact that GAstV-1 capsid proteins are

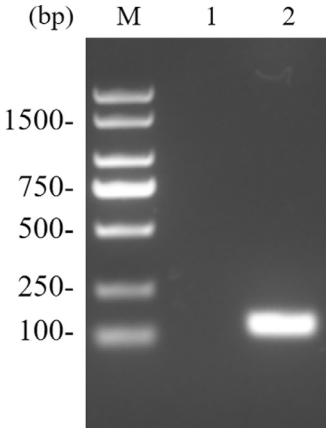

Figure 7. Identification of the virus re-isolated in goose embryos by reverse transcription-PCR. Total RNA was extracted from the allantoic fluids of normal goose embryos (1) and from goose embryos inoculated with the tissue homogenates of goslings that died of gout as part of the disease replication experiment (2). The extracted total RNA was analyzed via reverse transcription-PCR using GAstV-1 specific primers.

shorter (80 kDa) than mammalian astrovirus capsid proteins (approximately 90 kDa).

The pathogenicity of GAstV-2 has been confirmed, but the relationship between GAstV-1 and goose gout remains unclear. In the goose regression experiment, infected goslings showed clinical symptoms similar to those in the field and a significant peak of death at d 7 to 10, resulting in a mortality rate of 41.7%. Histopathological results revealed the presence of protein-like substances in the renal tubules, indicating that TZ03 strain infection caused increased permeability of the kidney epithelial barrier. It has been reported that astroviruses can increase the permeability of epithelial cells, and that increased permeability of kidney epithelial cells may contribute to gout disease (Moser et al., 2007; Bulbule et al., 2013).These data are similar to those observed in the field and the results of GAstV-2 infection experiments, indicating that the GAstV-1 strain TZ03 is the other causative agent of goose gout. Interestingly, encephalitis lesions were observed in the dead goslings, and TZ03 RNA was detected in the brain tissue (data not shown), which has rarely been reported in the field. Because many cases of astroviruses are associated with meningitis in mammals, the GAstV-1 TZ03 strain should be further investigated (Deiss et al., 2017).

In conclusion, we isolated GAstV-1 TZ03 from tissue samples of dead goslings characterized as having typical gout symptoms, and TZ03 was confirmed to be the causative agent of the disease. Whole-genome sequencing revealed that GAstV-1 TZ03 possesses certain distinct genomic characteristics and is highly divergent from GastV-2. Although the clinical symptoms of goose gout caused by GastV-1 TZ03 are similar to those caused by GastV-2, the differences in the replication ability and antigenic characteristics between GAstV-1 and GAstV-2 suggest that a more detailed investigation should be performed.

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DISCLOSURES

The authors have no competing interests to declare.

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