

Table S1. Primers used for the amplification of full-length genomes of the GAsV strain ZJ CX.

Primers	Sequences (5'-3')	Amplicon size (bp)	Purpose
GAsV 1F	CCGAAACAGCGATATGGCGGCCGGTG	2266	Genome sequencing
GAsV 2246R	GTCCTCTCGCAATTCCTCAA		
GAsV 1976F	CTGGCTGGTGTATTATTGATG	1198	Genome sequencing
GAsV 3154R	GACCATCAAACAATGAGTGCAT		
GAsV 3049F	GAGCAGCGCAAAAGGATCTG	1498	Genome sequencing
GAsV 4529R	TAAGACCACAGAAAGTCATA		
GAsV 4440F	GACACGAATGTCGTCATAG	2698	Genome sequencing
GAsV 7119R	CTTAAAAATCACATTTGATTC		
GAsV 6795F	GACTTCCACCTAGCAGTCTC	NA	3'RACE
GAsV 6838F	AAGAAGAGGCTGAGTACTGGA	NA	nest PCR
GAsV 412R	GTTGGACAAGAGCTCCACATCG		5'RACE
GAsV 204R	GCATTACACTTCATGAGAGCA	NA	nest PCR
GAsV II-F	GAGCAGGACCAGAATGAGAAA		qRT-PCR
GAsV II-R	CACCACCAATGAGCCTAGATAC		qRT-PCR
β -actin-F	ACATCAGGAAGGACCTGTACG		
β -actin-R	GGGGCGATGATCTTGATC		qRT-PCR
OASL-F	CAGCGTGTGGTGGTTCTC		
OASL-R	AACCAGACGATGACATACAC		qRT-PCR
TLR-3-F	CTCTCTGGAAAAAATAAGCAATTTG		
TLR-3-R	CTCAAGCAAAGTGCATGATTGC		qRT-PCR
TLR-7-F	CACAGAAAAATGGTACCTC		
TLR-7-R	TACATCGCAGGGTAAACT		qRT-PCR
IFN- α -F	CAGCACCACATCCACCAC		
IFN- α -R	TACTTGTTGATGCCGAGGT		qRT-PCR
Mx2-F	TTCACAGCAATGGAAAGGGA		
Mx2-R	ATTAGTGTCGGGTCTGGGA		qRT-PCR
IL-6-F	AAGTTGAGTCGCTGTGCT		
IL-6-R	GCTTTGTGAGGAGGGATT		qRT-PCR
IL-8-F	GCTGTCCTGGCTCTTCTCCTGATT		
IL-8-R	GGGTCCAAGCACACCTCTCTGTTG		qRT-PCR
IL-10-F	TGCCTCCACTTGTCTGACC		
IL-10-R	TCCTCCATGTAGAACCGCATC		qRT-PCR
IFITM1-F	ATCAAGGCCCGAGATAGGA		
IFITM1-R	AGAGTATCACCAGGACCAGAA		qRT-PCR
TRIM25-F	CTGGATTTCAACACAGCTCATAAC		
TRIM25-R	CCCAGCACTTGGGAACAATA		

Table S2. Analysis of the main functional amino acid sites between ZJCX and other GAsV II ORFs.

Protein	Position	Isolates							
		ZJCX	AH02	GD	JSCZ15	JSW10	ZJLD	G576	Others
ORF1a	175	M	I	M	I	M	M	M	M/I
	188	A	T	T	T	T	T	T	T
	265	T	T	S	S	S	S	S	S
	355	I	I	V	V	V	I	V	V/I
	428	N	N	T	T	N	N	T	N/T
	520	T	T	A	A	T	T	A	T/A
	527	V	A	A	A	A	A	A	A
	528	T	T	I	I	T	T	I	T/I
	535	R	R	S	S	R	R	S	R/S
	555	V	V	V	V	I	I	V	I/V
	890	V	A	A	A	A	A	A	V/A
	910	P	Q	Q	Q	Q	Q	Q	Q
	911	K	E	E	E	E	E	E	E
	912	G	V	V	V	V	V	V	V
	918	G	D	D	D	D	D	D	D
	927	V	A	A	A	A	A	A	A
ORF1b	30	T	I	I	I	I	I	I	I
	167	R	K	K	K	K	K	K	K/R
	415	R	K	K	K	K	K	K	K
ORF2	229	P	Q	Q	Q	P	P	P	P/Q
	289	A	A	A	T	T	A	T	A/T
	456	E	E	E	E	D	D	D	D/E
	464	N	N	A	A	N	N	N	A/N
	498	L	L	I	I	I	I	I	L/I
	540	L	L	L	L	Q	Q	Q	L/Q
	586	T	S	S	T	S	S	S	T/S
	587	D	N	N	N	N	N	N	N
	610	G	E	E	E	E	E	D	E/D
	614	A	A	A	A	T	A	N	A/N/S/T
	634	D	E	E	D	E	E	E	D/E
	695	T	A	A	A	A	A	A	A

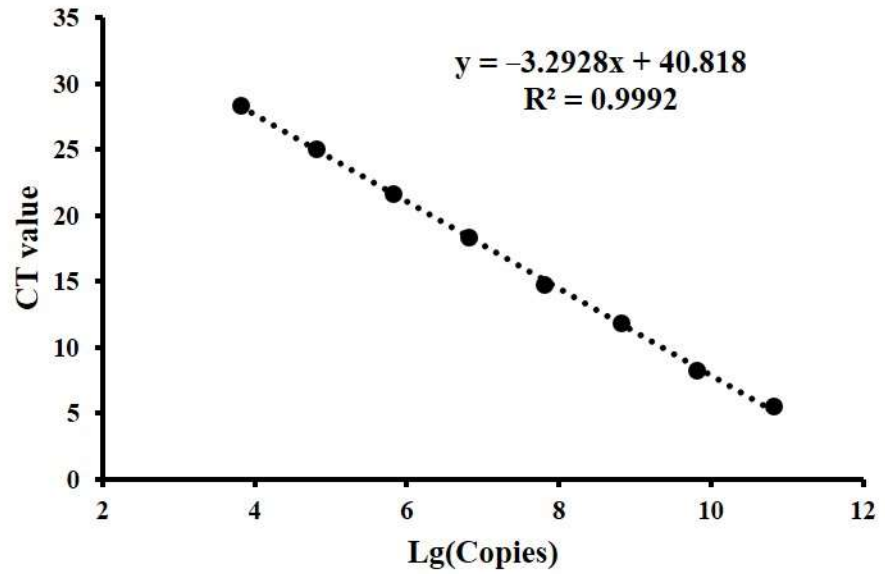


Figure S1. Establishment of a standard curve for the Duplex RT-qPCR assay. The RdRp gene fragment was amplified by PCR using GAstV-II cDNA template. The PCR product was purified and linked to the pEASY-T5 vector. DNA concentrations were determined and the copy number per μL was calculated according to the formula: $6.02 \times 10^{23} \text{ copies/mol} \times \text{Concentration/Average molecular weight}$. The recombinant standard plasmid with $6.49 \times 10^{10} \text{ copies}/\mu\text{L}$ was 10-fold diluted before use. One Step PrimeScript III RT-qPCR Mix was used according to instruction. The standard curve was obtained according to the average threshold cycle (Ct) and the logarithm of the initial copy number.



Figure S2. Isolation and identification of GAstV in goose embryos. The pathological changes in goose embryos infected with GAstV in the presence of haemorrhage and oedema.

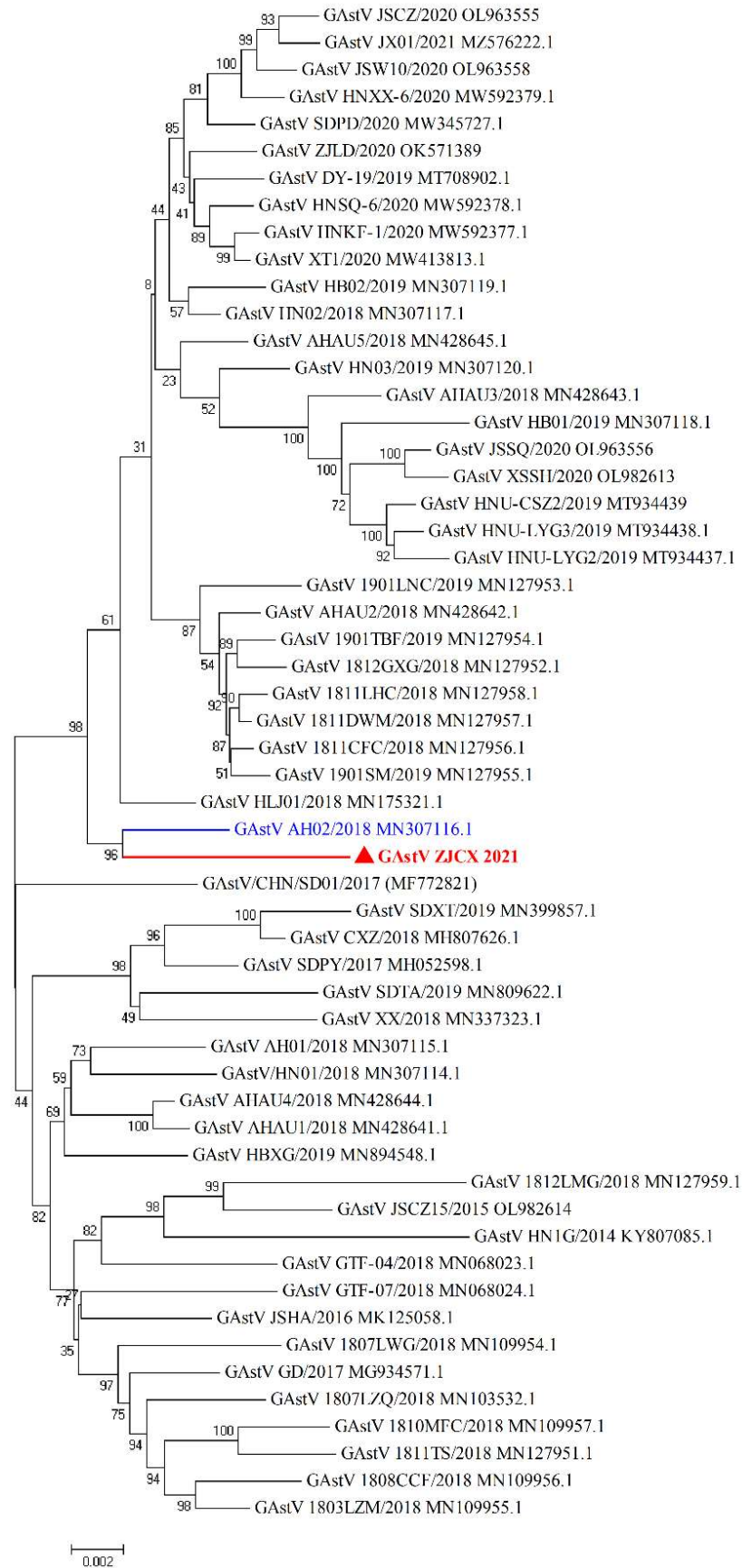


Figure S3. Phylogenetic tree of most GAsV II strains in China based on whole-genome sequences. Phylogeny-based genotyping of GAsV strains, constructed based on ClustalW alignment as a neighbor-joining tree via the neighbor-joining method (1,000 bootstrap replicates). The ZJCX (red color) strain get close to AH02 (blue color) in genetic relationships.