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Highly Pathogenic Swine Getah Virus in Blue Foxes, Eastern China, 2017

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We isolated Getah virus from infected foxes in Shandong Province, eastern China. We sequenced the complete Getah virus genome, and phylogenetic analysis revealed a close relationship with a highly pathogenic swine epidemic strain in China. Epidemiologic investigation showed that pigs might play a pivotal role in disease transmission to foxes.

Getah virus (GETV; genus *Alphavirus*, family *Togaviridae*) is a mosquito-borne RNA virus that causes death in young piglets, miscarriage in pregnant sows, and mild illness in horses (1–3). Serologic surveys show that the infection might occur in cattle, ducks, and chickens (4); some evidence suggests that GETV can infect humans and cause mild fever (5,6).

In September 2017, twenty-five 5-month-old blue foxes at a farm in Shandong Province in eastern China showed symptoms of sudden fever, anorexia, and depression; 6 of the 25 animals had onset of neurologic symptoms and died on the third day of illness. We collected blood samples from 45 healthy and 25 ill foxes. We subjected the tissue samples from dead animals, including the brains, lungs, spleens, kidneys, livers, intestines, hearts, and stomachs, to hematoxylin and eosin staining. Microscopic examination confirmed the

presence of typical lesions in cerebral cortices with mild neuronal degeneration and inflammatory cell infiltration in vessels, as well as severe hemorrhagic pneumonia, congestion, and hemorrhage with a large number of erythrocytes in the alveolar space (Figure) (1). No obvious lesions were found in other organs.

We used supernatants of homogenized brain and lung tissues from each dead fox to inoculate Vero cells, as described previously (7). We observed a cytopathogenic effect within 72 hours. We observed numerous spherical, enveloped viral particles, ≈ 70 nm in diameter, after negative staining in a transmission electron microscope. To identify potential viral pathogens, we performed reverse transcription PCR (RT-PCR) to detect a panel of viruses, including canine distemper virus, canine parvovirus, canine coronavirus, and canine adenovirus. However, we detected none of these classical endemic viruses.

During the investigation, farmers reported that the foxes had been fed on organs from symptomatic pigs. We therefore tested for the presence of African swine fever virus, pseudorabies virus, porcine reproductive and respiratory syndrome virus, classical swine fever virus, Japanese encephalitis virus, porcine circovirus type 2, porcine circovirus type 3, porcine cytomegalovirus, and alphavirus by using the primers for those viruses (Appendix Table 2, <https://wwwnc.cdc.gov/EID/article/25/6/18-1983-App1.pdf>). RT-PCR using universal primers for alphavirus (M2w-cMw3) produced a 434-bp amplicon when we tested all samples from dead foxes. Sanger sequencing of the amplicon and a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) identified the sequence as that of GETV.

To further investigate the epidemic GETV infection, we performed quantitative RT-PCR by using RNA from all fox samples, as described elsewhere (7). Lung samples from all 6 dead foxes were positive, whereas only 2 samples from the remaining 19 ill foxes were also positive. None of the samples from healthy foxes were positive (Appendix Tables 1, 3). We measured serologic neutralizing antibodies by using a GETV isolate from a symptomatic fox, as previously described (8,9). Results showed no neutralizing antibody ($<1:2$) in healthy blue foxes (group 1) and variable levels of neutralizing antibodies (1:2 to 1:256) in ill foxes (groups 2–4) (Appendix Table 3). Samples from ill foxes with lower antibody titers had higher copies of RNA (groups 2–4). Spearman correlation analysis revealed a significant negative correlation between antibody titers and viral RNA copy numbers ($r^2 = 0.952$; $p < 0.01$).

We obtained the complete genome of the novel GETV SD1709 strain (GenBank accession no. MH106780) by using a conventional RT-PCR method (10). SD1709 genome sequence comparisons showed high identity with the porcine GETV strain (HuN1) at the nucleotide (99.6%) and deduced amino acid (99.7%–99.8%) sequences (Appendix Table 4). Furthermore, phylogenetic analysis of the

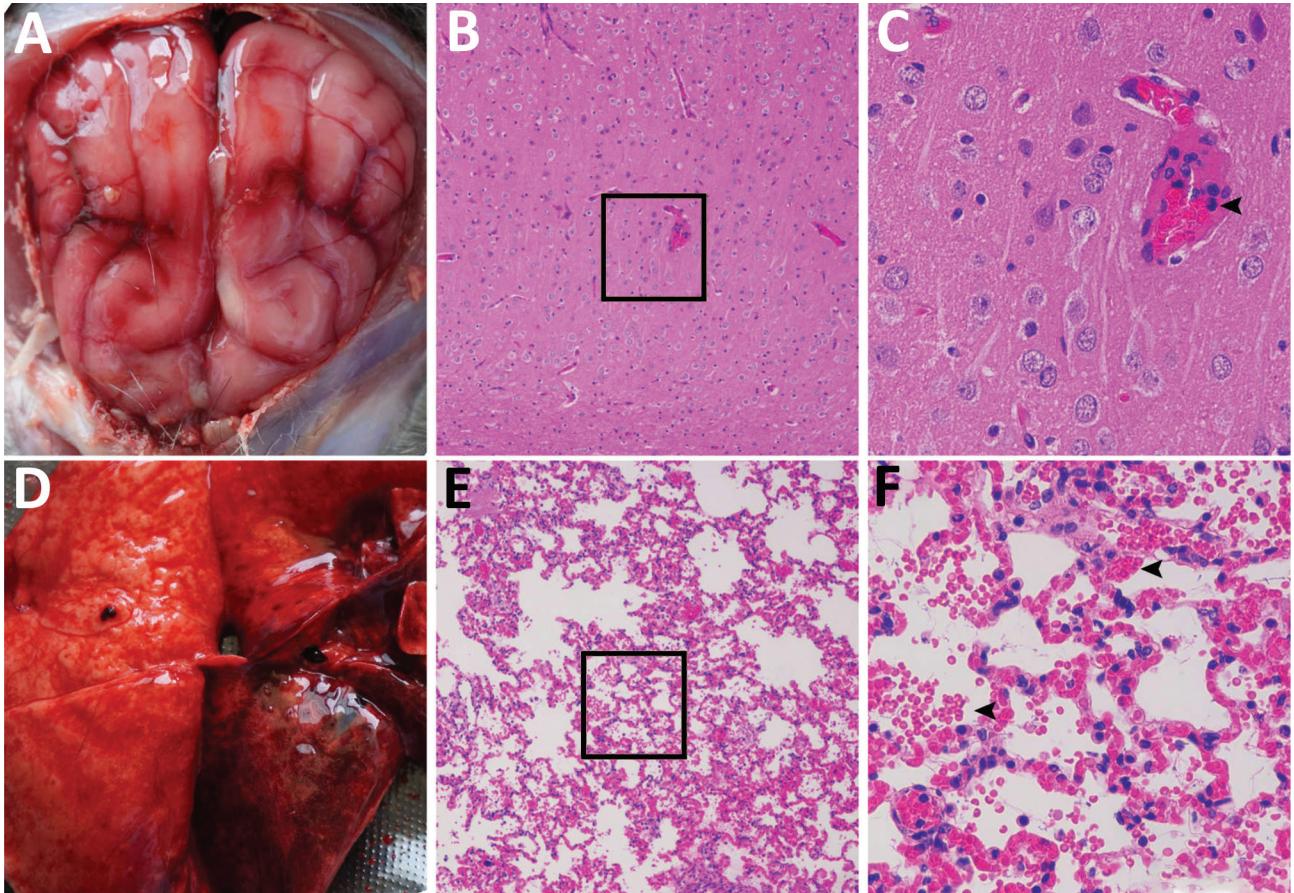


Figure. Dissected brain and lung of a dead fox, collected in 2017 in Shandong Province, eastern China, and histopathologic examination of samples using hematoxylin and eosin staining. A) Brain, showing congestion in the meninx. B) Histologic view of meninx, showing mild neuronal degeneration and inflammatory cell infiltration in vessels. Original magnification $\times 100$. Box indicates area enlarged in panel C. C) A higher magnification view (original magnification $\times 400$) of lesions in panel B, showing inflammatory cell infiltration in a vessel (arrow). D) Lung tissue, showing extensive congestion and hemorrhage. E) Histologic view of lung tissue, showing congestion, hemorrhage, or both, with many erythrocytes in the alveolar space. Original magnification $\times 100$. Box indicates area enlarged in panel F. F) A higher magnification view (original magnification $\times 400$) of tissue lesions in panel E, showing erythrocytes in the alveolar space (arrows).

complete genome and structural protein E2 gene indicated that the SD1709 strain was most similar to the recent epidemic HuN1 strain, which had caused large numbers of piglet deaths, stillbirths, and fetal mummies in southern China in 2017 (1) (Appendix Figures 1, 2).

We also detected GETV infection in pig serum samples and in mosquitoes (*Culex tritaeniorhynchus*, *Anopheles sinensis*, and *Armigeres subalbatus*) collected in the same region. The infection rate in pigs detected by quantitative RT-PCR was 20.0% (4/20) and by serum neutralization was 75.0% (15/20). The minimum infection rate in mosquitoes was $\approx 1.09\%$; *C. tritaeniorhynchus* mosquitoes had a higher minimum infection rate (2.31%) compared with other mosquito species (0–0.80%). These results suggest that pigs and *C. tritaeniorhynchus* mosquitoes might play a role in transmitting highly pathogenic GETV to captive foxes in this region (Appendix Tables 5, 6).

In China, the disease caused by GETV has only been reported in pigs in Hunan Province, although the virus has been detected in mosquitoes in >10 provinces (1,4). Our study provides evidence that GETV can cause lethal infection in blue foxes. Investigation of transmission routes for GETV in animals might help to prevent outbreaks of GETV disease in China.

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The affiliation for Marie E. Killerby was listed incorrectly in Risk Factors for MERS-CoV Seropositivity among Animal Market and Slaughterhouse Workers, Abu Dhabi, United Arab Emirates, 2014–2017 (A. Khudhair et al.). Dr. Killerby is affiliated only with the Centers for Disease Control and Prevention (Atlanta, GA, USA). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/25/5/18-1728_article).

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Appendix

Appendix Table 1. Analysis of a Getah virus–infected blue foxes with Neurologic symptoms and pneumonia, China, 2017*

Sample material	Histopathologic finding	Real-time PCR (cycle threshold)†
Brain	Mild neuronal degeneration and inflammatory cells, infiltrate in vessel	– (>35)
Lung	Severe congestion and hemorrhage developed in capillary of alveolar septa, many erythrocytes observed in alveolar space	+ (26–30)
Spleen	NSML	– (>35)
Kidney	NSML	– (>35)
Liver	NSML	– (>35)
Intestine	NSML	– (>35)
Heart	NSML	– (>35)
Stomach	NSML	– (>35)

*NSML, no major microscopic lesions; –, negative; +, positive

†Negative result >35; positive result <35.

Appendix Table 2. Primers used in the present study

Virus	Primer name	Anneal site	Sequence(5'–3')	Length of amplification	References*
CDV	F4854	Fsp	TCCAGGACATAGCAAGCCAACA	681 bp	(1)
	R5535		GGTTGATTGGTTTCGAGGACTGAA		
CPV	555for	VP2	CAGGAAGATATCCAGAAGGA	583 bp	(2)
	555rev		GGTGCTAGTTGATATGTAATAAACA		
CCoV	CCoV-F	Hel	ACATGGTATATCTATGTGCGCAA	252 bp	(3)
	CCoV-R		TGCAAGGCGCACTTGAGAT		
CAV	CAV-F	E1A	TGTGCCATCGACAAGGAA	433 bp	(3)
	CAV-R		CTAATAGAAGCGGCCAACTG		
ASFV	CD2–2F	EP402R	TCTGTTGATCCCAACTATTACA	816 bp	(4)
	CD2–2R		ATGGCGGGATATTGGGTAGT		
PRV	PRV-F	gD	ATGCGGCCCTTTCTG	217 bp	(5)
	PRV-R		CGGTTCTCCCGGTATTTAAGC		
PRRSV	ORF5-F	ORF5	GGCGACCGTTTTAGCCTGTCTT	735 bp	(6)
	ORF5-R		ATCATTATTGGCGTGTAGGTG		
CSFV	CSFV1	E2	GCTCCTGGTTGGTAACCTCGG	508 bp	(7)
	CSFV2		TGATGCTGTCACACAGGTGAA		
JEV	JEV1	E	TGTGGACTTTTCGGGAAGGG	1015 bp	(7)
	JEV2		GGTGAACGGCTCTTCCTATG		
PCV2	F-PCV	ORF1	GCTGCCACATCGAGAAAG	565 bp	(8)
	R-PCV		GACAGCAGTTGAGGAGTACC		
PCV3	PCV3-F	Cap	TCCAAACTTCTTTCGTGCCGTAG	264 bp	(9)
	PCV3-R		GGCTCCAAGACGACCCTTATGC		
PCMV	PCMV-F	gB	CCCTGATCTTAAATGACGAGGACGTGAC	413 bp	(8)
	PCMV-R		ACCGTCTGAGAGACTGAACCTCTCTGACAC		
Alphavirus	M2w	NS1	YAGAGCDTTTTCGCASTRGCHW	434 bp	(10)
	cMw3		ACATRAANKGNGTNGTRTCRAANCCDAYCC		
	M2W2		TGYCCNVTGMDNWSYVCNGARGAYCC		

*Primer sequences used to amplify the several important virus infected Canidae and pigs in previous reports.

Appendix Table 3. RT-qPCR and Serum neutralization (SN) tests results of GETV in serum samples of blue foxes from Shandong, eastern China*

Groups†	Clinical symptoms	SN test results (no. samples)‡	RT-qPCR (copies/ μ L)‡
1	No symptoms	<1:2 (n = 45)	Negative
2	Fever, depression, anorexia, systemic neurologic symptoms, dyspnea, and emesis; weak in appearance; ultimately died	1:2 (n = 1)	1.698 $\times 10^3$
		1:2 (n = 1)	1.445 $\times 10^3$
		1:2 (n = 1)	1.718 $\times 10^3$
		1:4 (n = 1)	4.266 $\times 10^2$
		1:8 (n = 1)	1.466 $\times 10^2$
3	Fever, depression, anorexia	1:16 (n = 1)	4.764 $\times 10^1$
		1:16 (n = 1)	6.531 $\times 10^0$
		1:32 (n = 1)	Negative
		1:32 (n = 1)	Negative
		1:32 (n = 1)	Negative
		1:32 (n = 1)	Negative
4	Spontaneous clearance	1:64 (n = 6)	Negative
		1:128 (n = 5)	Negative
		1:256 (n = 1)	Negative

*RT-qPCR, quantitative reverse transcription polymerase chain reaction; SN, serum neutralization

†The collected samples were divided into Group 1 (<1:2), Group 2 (1:2–1:16), Group 3 (1:16–1:64), Group 4 (>1:64) and according to the neutralizing antibody titer >1:4 was positive.

‡Spearman correlation analysis showed significant negative correlation between the antibody titers and viral RNA copy numbers ($r^2 = 0.952$, $p < 0.01$).

Appendix Table 4. Nucleotide and amino acid sequence identity (%) for the complete genomes between the isolates SD1709 from fox in this study and others

Virus isolates	Complete genome (nt)	SD1709, %			
		Nonstructural polyprotein		Structural polyprotein	
		nt	aa	nt	aa
12IH26	97.7	97.7	99.4	97.7	99.4
14-I-605-C1	97.7	97.6	99.3	97.7	99.4
14-I-605-C2	97.7	97.6	99.3	97.7	99.4
15-I-1105	97.6	97.6	99.1	97.6	99.3
15-I-752	97.7	97.6	99.2	97.7	99.4
16-I-599	97.7	97.6	99.2	97.6	99.3
16-I-674	97.6	97.6	99.1	97.6	99.3
16-I-676	97.6	97.6	99.1	97.6	99.3
GETV-V1	97.8	97.8	99.3	97.6	99.2
HB0234	97.8	97.8	99.1	97.6	99.0
HuN1	99.6	99.5	99.7	99.7	99.8
Kochi/01/2005	99.4	99.4	99.7	99.3	99.5
LEIV 16275 Mag	97.5	97.5	99.4	97.3	99.0
LEIV 17741 MPR	98.5	98.4	99.4	98.5	99.4
M1	97.9	98.0	99.1	97.7	98.3
MI-110-C1	98.5	98.4	99.6	98.5	99.4
MI-110-C2	98.5	98.4	99.6	98.5	99.4
ROK	98.1	98.1	99.6	98.1	99.4
Sagiyama virus	97.2	97.4	99.2	96.8	98.2
SC1210	97.5	97.8	99.4	97.6	99.2
YN0540	97.6	97.9	99.4	97.8	99.4
YN12031	96.3	96.3	98.8	96.1	98.2

Appendix Table 5. RT-qPCR and SN were used to detect pigs serum positive rates of GETV on different age group*

Sampling age group	RT-qPCR			SN		
	No. swine	No. swine testing positive	No. (%) of swine testing positive (95%CI)	No. swine	No. swine testing positive	No. (%) of swine testing positive (95%CI)
Nursery pigs	8	2	25.0 (23–26)	8	5	62.5 (41–83)
Fattening pigs	5	1	20.0 (18–21)	5	3	60.0 (15–104)
Sow	7	1	14.3 (13–15)	7	7	100.0 (54–145)
Total	20	4	20.0 (19–21)	20	15	75.0 (48–101)

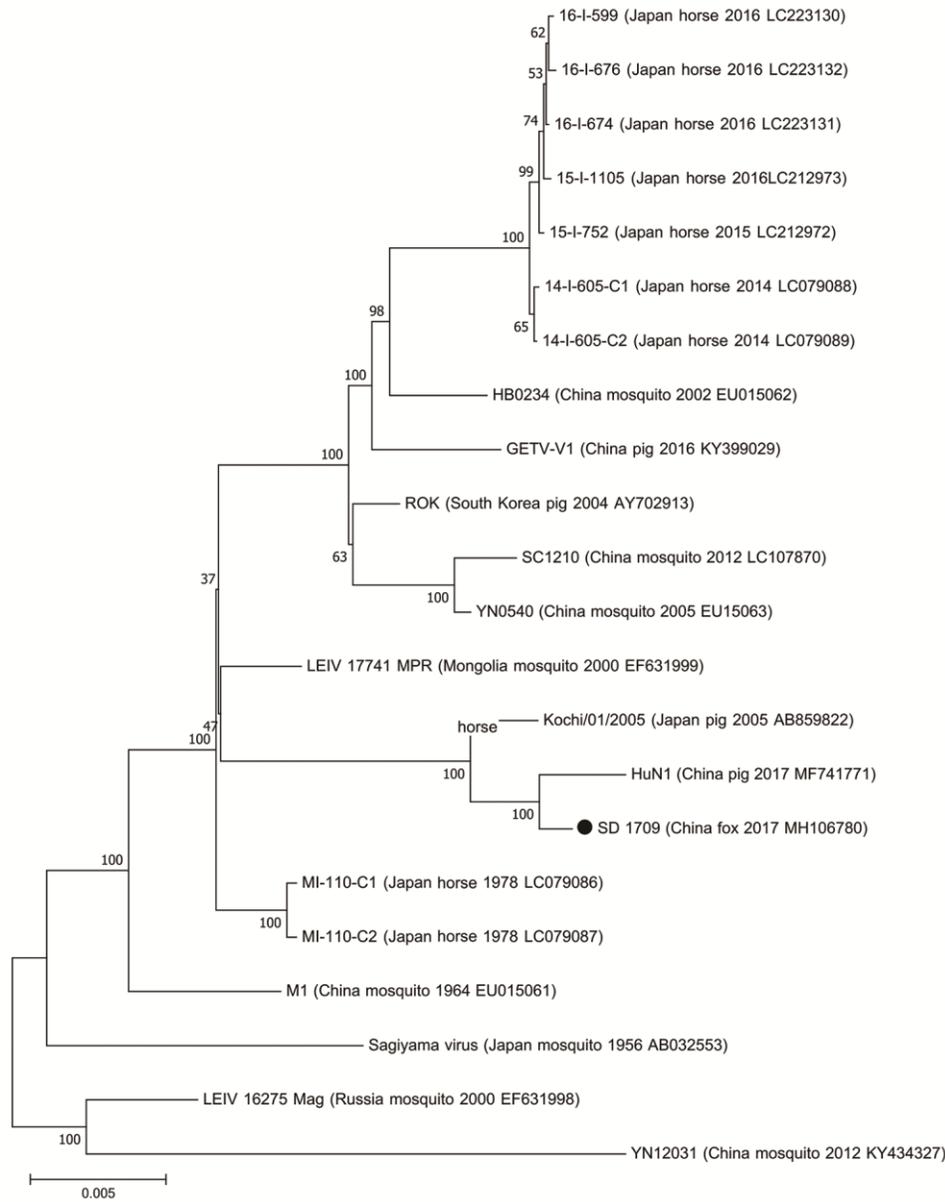
*RT-qPCR, quantitative reverse transcription polymerase chain reaction; SN, serum neutralization.

Appendix Table 6. GETV infection in mosquitoes collected from Linyi of Shandong province, eastern China by RT-qPCR*

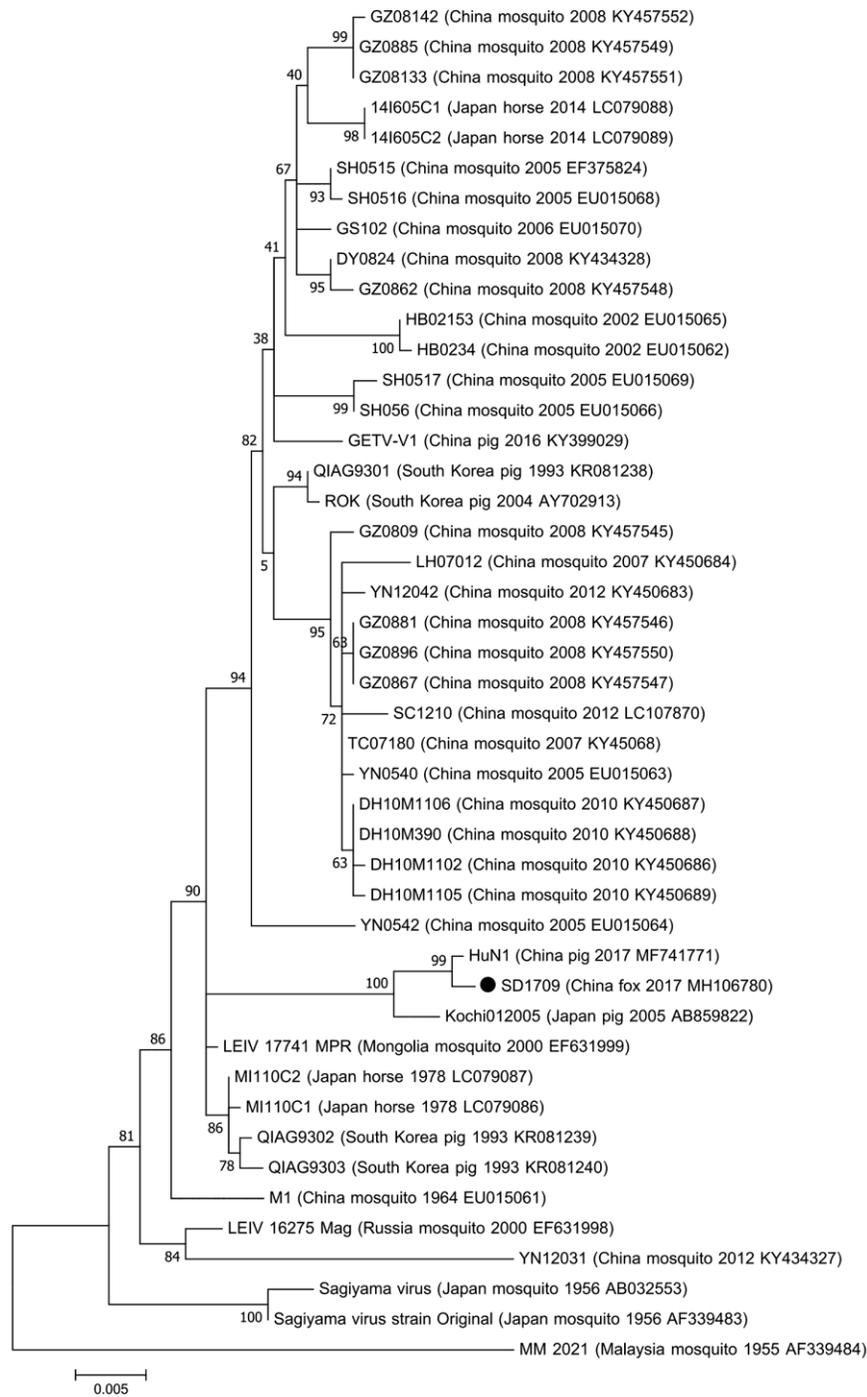
Species	No. mosquitoes	No. pools (100 mosquitoes/pool)	MIR of mosquitoes, % (no. positive pools/total specimens)†
<i>Culex tritaeniorhynchus</i>	1,300	13	2.31 (3/1300)
<i>Anopheles sinensis</i>	2,500	25	0.80 (2/2500)
<i>Armigeres subalbatus</i>	800	8	0.00 (0/800)
Total	4,600	46	1.09 (5/4600)

*RT-qPCR, quantitative reverse transcription polymerase chain reaction; MIR, minimum infection rate.

†MIR uses the assumption that a positive pool contains only 1 infected mosquito the minimum infection rate, which is calculated: ((number of positive pools/total specimens tested) x 1,000) (<https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html>).



Appendix Figure 1. Phylogenetic analyses of the nucleotide sequences of the complete genome of Getah virus isolated in Shandong. Evolutionary history was inferred using the maximum likelihood method with the Tamura–Nei model and gamma-distributed rate heterogeneity in MEGA 7. The percentage of replicates in which the associated virus clustered together in the bootstrap test (1,000 replicates) is shown next to the branch in each tree. The strain isolated in this study is identified by ●. The percentage bootstrap support is indicated by the value at each node. Scale bar denotes nucleotide substitutions per site.



Appendix Figure 2. Phylogenetic analyses of E2 gene nucleotide sequences of Getah virus isolated in Shandong, 2017. The strain isolated in this study is identified by ●.

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