Human Tacheng Tick Virus 2 Infection, China, 2019

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We used metagenomic analysis to identify Tacheng tick virus 2 infection in a patient with a history of tick bite in northwestern China. We confirmed the virus with reverse transcription-PCR, virus isolation, and genomic analysis. We detected viral RNA in 9.6% of ticks collected from the same region.

Emerging pathogenic tickborne viruses have attracted much attention because of the increasing incidence of tickborne viral diseases and their effects on human health (*1*–*4*). In 2015, high-throughput sequencing of samples from ticks in China revealed several novel phleboviruses, including Tacheng tick virus 2 (TcTV-2), Changping tick virus 1, Bole tick virus 1 (BlTV-1), Lihan tick virus, Yongjia tick virus 1, and Dabieshan tick virus (*5*). However, the risk for human infection from these viruses is not yet known. We report on TcTV-2 infection in patient in China and describe methods for virus isolation and genomic analysis.

The Study

The patient was a 38-year-old man who lived in northwestern China and had frequent contact with horses and sheep. On May 29, 2019, he noticed a tick embedded on his left upper arm and removed it himself. He noted a localized rash with slight pain and discomfort. On June 16, fever developed and soon after the patient had chills, severe fatigue, headache, anorexia, nausea, and vomiting. On June 20, he was admitted to the local hospital with a temperature of 37.9°C, which increased to 39.5°C the next day. The patient was initially given intravenous cefotaxime sodium and levofloxacin for 3 days for suspected tickborne bacterial disease, but these treatments did not alleviate his symptoms.

On June 24, the patient was admitted to the First Affiliated Hospital of Medical College of Shihezi University in Shihezi. Physical examination showed erythema at the bite site (Figure 1, panel A) and neck stiffness. Cerebrospinal fluid (CSF) analysis showed a total of 1.07×10^8 nucleated cells (92% hyaline leukocytes and 8% pleocaryocytes), an increased protein level (0.99 g/L), and decreased levels of CSF glucose (2.3 mmol/L) and chloridion (116.0 mmol/L). The patient was given intravenous ceftriaxone for 12 days, but still experienced headache, nausea, and vomiting, and his erythema was not decreasing.

Blood, throat swabs, urine, and CSF samples were obtained from the patient on days 9, 16, and 40 after illness onset. We tested the patient samples by PCR or reverse transcription-PCR (RT-PCR) for potential tickborne pathogens, including severe fever with thrombocytopenia syndrome virus, tickborne encephalitis virus, *Borrelia burgdorferi* sensu lato, *Anaplasma*, *Babesia*, *Rickettsia* spp., Tacheng tick virus 1, TcTV-2, Tacheng tick virus 5, BlTV-1, and Bole tick virus 4 (*2*). We detected TcTV-2 by metagenomic analysis on blood collected on day 9 and confirmed the virus by RT-PCR targeting the large (L) gene (Appendix Tables 1, 2, https://wwwnc.cdc.gov/EID/article/27/2/19-1486-App1.pdf). We detected TcTV-2 in blood, throat swabs, and urine samples from the patient. We ruled out bacterial infection in blood and CSF by using routine culture methods and 16S rRNA gene broad-range PCR, which confirmed that no bacterial infection occurred in this patient.

On July 18, the patient was admitted to the hospital again. He was given intravenous acyclovir for 12 days and his clinical symptoms and erythema vanished without any sequelae (Appendix Table 3).

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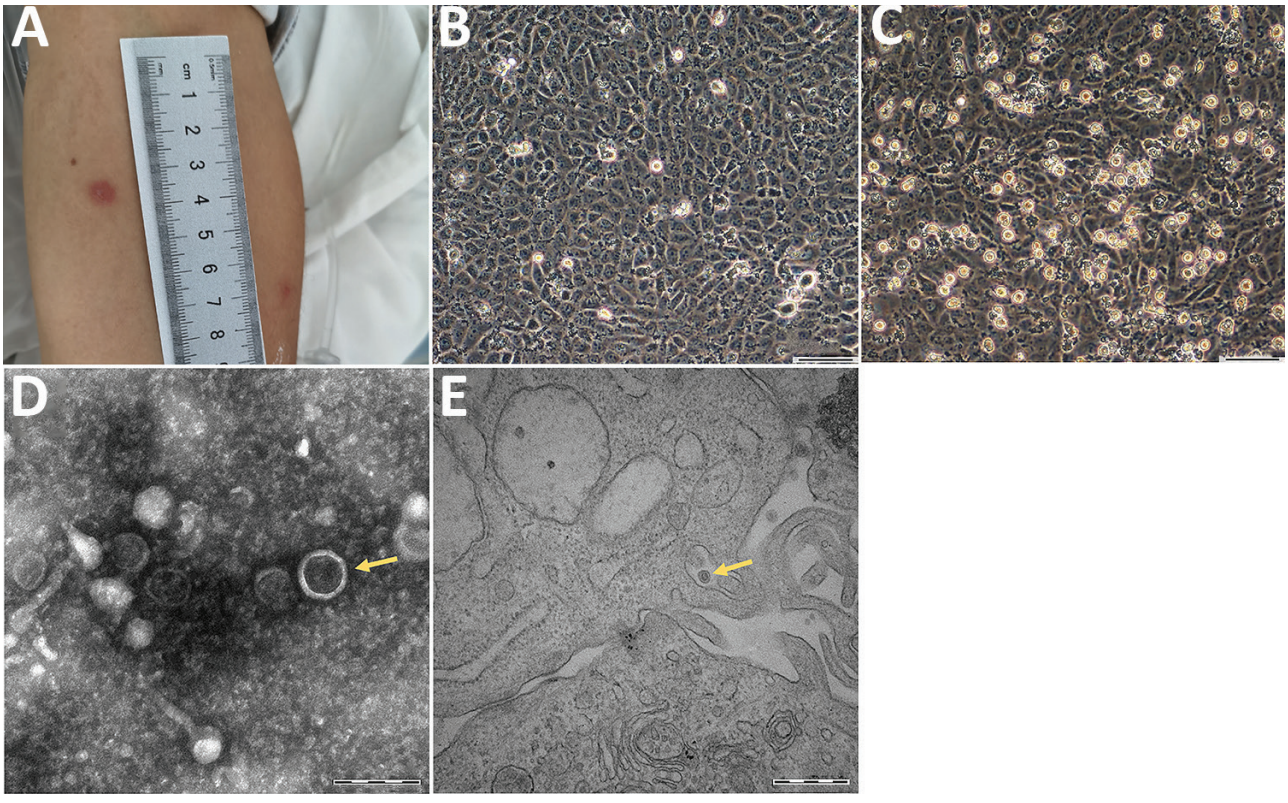

Figure 1. Clinical and morphological features of Tacheng tick virus 2 in a patient, China. A) Erythema at the site of tick bite on the anterior surface of the patient's left arm. B) Human hepatocellular carcinoma (SMMC-7721) cells without TcTV-2 infection; magnification × 100. Scale bar indicates 50 μm. C) TcTV-2–infected SMMC-7721 cells showing cytopathic effects visible by light microscopy; magnification × 100. Scale bar indicates 50 μm. D) Negatively stained virions purified from TcTV-2–infected SMMC-7721 cells (arrows); magnification × 25,000. Scale bar indicates 200 nm. E) Transmission electron microscopy image of TcTV-2–infected SMMC-7721 cells (arrows); magnification × 50,000. Scale bar indicates 500 nm. TcTV-2, Tacheng tick virus 2.

To isolate the virus, we inoculated human hepatocellular carcinoma (SMMC-7721) cells, African green monkey kidney (Vero) cells, baby hamster kidney cells, and human foreskin fibroblasts with the serum samples collected during early illness onset (Appendix Figure 2). We performed electron microscopy analysis on infected cells showing cytopathic effect, as described previously (*6*). After incubation, only the SMMC-7721 cells demonstrated cytopathic effect associated with TcTV-2 after several passages (Figure 1, panels B,C; Appendix Figure 3). The virions were spherical with a diameter of ≈90–100 nm (Figure 1, panel D). The virions could be seen in the cytoplasm of infected SMMC-7721 cells on transmission electron microscopy (Figure 1, panel E). We tested for TcTV-2–specific antibodies by using immunofluorescence assay. Serologic detection showed that TcTV-2 IgM titer in serum samples decreased from 1:40 on day 9 to 1:10 on day 40 after illness onset, and IgG titer increased from 1:10 on day 9 to 1:80 on day 40 (Table).

Table. Results of immunofluorescence assay in detection of Tacheng tick virus 2 infection in a human, China*

Days post illness onset	Sample type	IFA titer	
		IgM	IgG
Day 9	Serum	1:40	<1:10
	Urine	<1:10	<1:10
	CSF	<1:10	<1:10
Day 16	Serum	1:20	1:10
	Urine	<1:10	<1:10
	CSF	<1:10	<1:10
Day 40	Serum	<1:10	1:80
	Urine	<1:10	<1:10
	CSF	<1:10	<1:10

*CSF, cerebrospinal fluid; IFA, immunofluorescence assay.

We isolated total RNA from infected cells and used the isolates to amplify the L and small (S) gene segment sequences by using primers based on our metagenomic analysis (Appendix Table 2, Table 4). The obtained L segment of TcTV-2 from the patient (GenBank accession no. MN567189) showed 98.8% (6,579/6,659) identity to the L segment of strain TC252 (GenBank accession no. KM817684) and the S segment

from the isolate (GenBank accession no. MN567190) showed 99.2% (2,169/2,185) identity to the S of strain TC252 (GenBank accession no. KM817744).

Phylogenetic analysis suggested that TcTV-2, together with Phlebovirus sp. 20A L, Pacific coast tick phlebovirus, Changping tick virus 1, BlTV-1, Lihan tick virus, Yongjia tick virus 1, Dabieshan tick virus, American dog tick phlebovirus, *Rhipicephalus*-associated phlebovirus 1, Xinjiang tick phlebovirus, tick phlebovirus, and brown dog tick phlebovirus 2 formed a separate branch (Figure 2; Appendix Figure 1). An M segment has yet to be detected in any of these viruses (*5*,*7*–*12*).

To identify local natural virus hosts in the environment, 345 adult ticks were collected in the area where the patient lived, including 108 *Dermacentor marginatus*, 183 *D. nuttalli*, 12 *D. silvarum*, and 42 *Hyalomma asiaticum*. We extracted total RNA of each tick and detected TcTV-2 by using RT-PCR with TcTV-2–specific primers (Appendix Table 1). Among 345

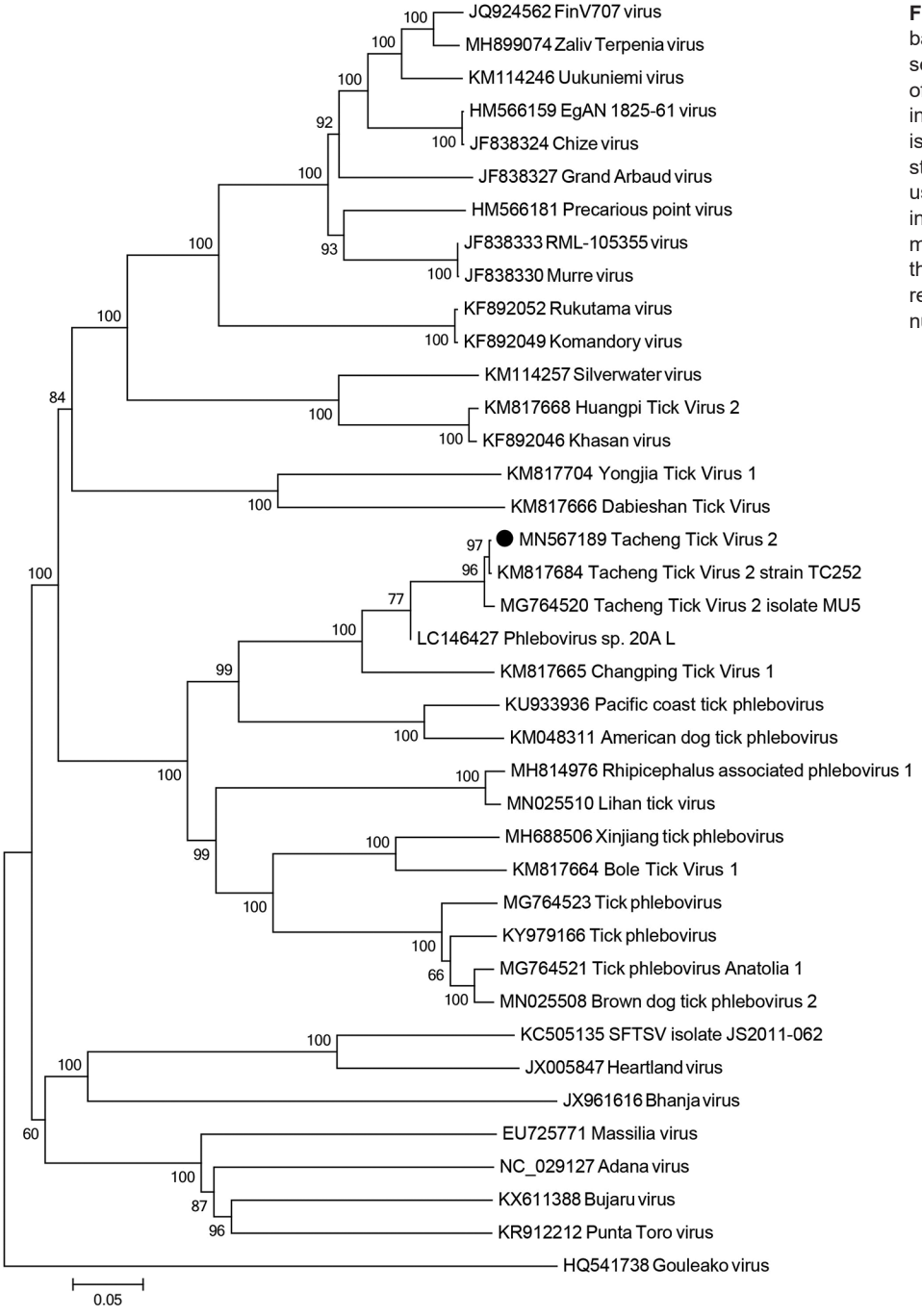

Figure 2. Phylogenetic analysis based on partial amino acid sequences of the L segment of tickborne viruses. Black dot indicates Tacheng tick virus 2 isolated from the patient in this study. The tree is constructed by using the neighbor-joining method in MEGA version 7.0 (https://www.megasoftware.net) and tested by the bootstrap method with 1,000 replications. Scale bar indicates nucleotide substitutions per site.

ticks, 33 (9.6%) carried TcTV-2. We noted high infection rates in *D. silvarum* (16.7%), *D. marginatus* (14.8%), *H. asiaticum* (11.9%), and *D. nuttalli* (5.5%). We obtained the partial fragments of the S segment of TcTV-2 in ticks and phylogenetic analyses showed that sequences from TcTv-2 in ticks were closely related to the isolate from the patient (Appendix Figure 1, Appendix Table 5).

We tried to obtain the medium (M) segment of TcTV-2 by designing a set of primers based on the conservative sequences of M segments from 15 typical phleboviruses (Appendix Table 6). We used these primers to amplify the M segment from both the patient and positive ticks detected by sequencing the L and S segments by using RT-PCR. We further analyzed the metagenomic sequences, but the results were negative.

Conclusions

Among currently known emerging tickborne phleboviruses, severe fever with thrombocytopenia syndrome virus and Heartland virus have been reported to infect humans and cause multiple organ damage, including to the liver and kidneys (*1*,*13*). In this study, TcTV-2 did not show any growth in Vero, human foreskin fibroblasts, or baby hamster kidney 21 cells, but had low level replication and growth in SMMC-7721 cells, indicating that the virus is not well adapted to mammals and likely is more common in arthropods than in mammals.

Transmission electron microscopy showed that TcTV-2 might harbor glycoprotein encoded by the M gene segment. The lack of M sequence data on homology-based approaches could indicate that insufficient homology exists between these viruses to detect the M gene in this manner. Sequencing methods that obtain a greater depth of coverage might help obtain the missing M sequences. To increase the virus titer and the likelihood of obtaining the M sequence, we recommend performing deep sequencing on the isolated virus.

TcTV-2 previously was identified in *D. marginatus* ticks from China (*5*) and in *H. marginatum* ticks from Turkey (*12*). We detected TcTV-2 in *D. nuttalli*, *D. silvarum*, *H. asiaticum* ticks and in blood, urine, and throat swab samples from a patient with febrile illness. Our findings suggest that person-to-person transmission might be possible through direct contact with body fluids or by droplet transmission. In addition, we noted more tick species found in northwest China that could act as TcTV-2 vectors (*14*), but this finding should be verified in further studies. Nonetheless, our study demonstrates that TcTV-2 could be emerging and infecting humans. Clinicians should consider TcTV-2 infections in patients with febrile illness and recent history of tick bites.

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About the Author

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Appendix

Appendix Table 1. Primer sequences of nested reverse transcription-PCR for detection of multiple tickborne pathogens in a study of Tacheng tick virus 2 in a patient, China, 2019

Targeting pathogens	Primer name	Primer sequence (5′→3′)	Gene segment	Product size, bp
Tacheng tick virus 2	F1	ATCTCCTCAACGGCAACTAT	359–812	454
	R1	GACATGCGGTTCTTCATTTT		
	F2	TCAACGGCAACTATGAGGAT	365–616	252
	R2	CTGGCTTGTATTGGAAGGAC		
Tacheng tick virus 5	F1	TGGTCAGATGGGAGGAAT	2105–2709	605
	R1	CCGCCCTAGCAAAGAAAT		
	F2	TATGGTTCCTCCAAAAGC	2243–2709	467
	R2	CCGCCCTAGCAAAGAAAT		
Bole tick virus 1	F1	GCAARCCTGGAAGTCAAGCC	4963–5754	812
	R1	AGAGCACCCGCAGCATAGTC		
	F2	GCAARCCTGGAAGTCAAGCC	4963–5703	741
	R2	CTCTGAGTGGGTTCCTGATT		
Bole tick virus 4	F1	ACGCTTGTAGGGCTCAGGTGTC	517–1079	563
	R1	CTGGTCGKCAATCTGRATGCAACA		
	F2	CTCAGGTGTCCTTTGACGATTAT	529–1079	551
	R2	CTGGTCGKCAATCTGRATGCAACA		

Appendix Table 2. Sequences of large gene contigs of Tacheng tick virus 2 in patient by metagenomic analysis and their identities to the reference strain*

Contig	Location	Sequence (5′→3′)	Sequence identity, no./No. (%)
1	252–372	TCCTCACCCTTCAAGCCTCAACTCACTTTTTCCATGATTTCACGTTCCAGTACC TGTCACGAGACACAGATGTTCCCTTCAAGAAATTCTATGAGACTGTGAATGAT GGCTTTGACAACCACACCCCTGATGTCATAATTGAAGACATCCAAGG	152/153 (99)
2	921–1101	GGATCTCAATTGGCTAGAATGCACCAAGAAGGTAAATGAGTACAGGACTTCCT TCCACAAGCCTCGCAAAATGCGAGGGAGGCTTTCACATAAATCAACTATCCC CTTCCCAGGCTTTATGCCCATGGTATCTGAATATCCTTCCATTAGCGTCAAGG AGATCCTCAAAAAACCCTCCAACTTCCCCCA	186/187 (99)
3	1668–1788	GGGGCCGGGTGAGTACATTATCAAGAAGCTGAGGAACTTTGAGGTCTTCCTG CTCATAAAGCCAACCAAGAGCAATGGGCCGATGTTCGTATCTCTGGCTTGGC ATGCAAAAGATGTGTCTAACACATACCTTACAGACTACAT	135/141 (96)
4	1708–1828	GTCTTCCTGCTCATAAAGCCAACCAAGAGCAATGGGCCGATGTTCGTATCTCT GGCTTGGCATGCAAAAGATGTGTCCAACACATACCTTACAGACTACATGGTGT TCAAGGCCACTCAGCAGGTGGGAGACTGGTGCTGGACTGAATTCCATTCCTT AAAACCATCCA	163/169 (96)
5	2088–2208	CGGGCTTTGTGTCAGATCCCTGTCTGCCCCATCCTCACAAGATGATCGAAAA GCTTCCAG ATGTGGCCAGGACCAAACTCCAGGTCTGGCTGATCAACAAGAGCCTCAACCT CATGCAAG ACATTGCTCTGAGGCCTTATCAGCCA	143/145 (99)
6	2238–2558	CCGCAGAATGCATTCAATACATCCATGTGCATTGATTGCACTATGTTGGGGCC CCAGGTTGCTTTGAGGTGCACCAGTGCCACGTCACACACGTACTTCTGAAAT GACACGGAGTACTCATGTGTCTTTATGTCGTCCAGAGGAGGATCCTTCCGGC CTA	159/160 (99)
7	4547–4667	CCACATATATGTCCAAAGAGGGTCAAAACGTGCAGGGTCGAAGGAAGACAGT CCAAACCAGGGTGGTGGTTTTCCACAGAGAAGAGGCAATGCGAGCAAGGCC AGAAGATGTCCTCACAGATGTATGGTGGGGTCGA	134/135 (99)
8	4677–4857	GGCGGGCCTCAGAAGGAGTGGCCTCACTTTGCCTGCACTGAAGGAGCATTTT GAGCAACTGCAGAGAGTGCTCCCATGGCTAACCAAGGATCCCAATGAGTCAT TGAGAAGATCCCCTTTCTTGCACCACCATCAACTCAGGAACTTTCTCTCGCGG ATGGAGATCCAAGGTAGAGAAGTCAGGCT	179/183 (98)

*GenBank accession number of the reference strain is KM817684.

Appendix Table 2. Sequences of N gene contigs of Tacheng tick virus 2 isolated in a patient by metagenomic analysis and their identities to the reference strain*

Contig	Location	Sequence (5′→3′)	Sequence identity, no./No. (%)
1	752–872	CCATGATTGTGCTCTACCTCACAAGGGGCACAAACACAGAAAAAATGAAGAACCGC ATGTCCGAGATGGGCAAGATGCTGATGGATCGGCTGGAAAAGCAGTACCAGATCC GGAAGGGTGCCGTGGCGCCCAAGGAGATCACCCTGGCCAGGGTTGCCCTCACCT ACCCGG	170/171 (99)
2	774–894	AAGGGGCACAAACACAGAAAAAATGAAGAACCGCATGTCCGAGATGGGCAAGATG CTGAT GGATCGGCTGGAAAAGCAGTACCAGATCCGGAAGGGTGCCGTGGCGCCCAAGGA GATCAC CCTGGCCAGGGTTGCCCTCACCTACCCGG	148/149 (99)

*GenBank accession number of the reference strain is KM81744.

Appendix Table 3. Diagnostic and treatment information for patient with Tacheng tick virus 2 infection, China, 2019*

Days post illness onset	Day 9				Day 16			Day 40			
			Throat				Throat			Throat	
Sample type	Blood	Urine	swab	CSF	Blood	Urine	swab	Blood	Urine	swab	CSF
Nested RT-PCR	+	+	+	–	+	+	+	+	+	+	–
Virus isolation	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Co-infection			–				–			–	
Laboratory findings, date	Jun 18		Jun 25		Jun 28		Jul 4		Jul 18		Jul 29
Blood											
Leukocyte count (×10^9/L)	11.62		10.9		NA		NA		5		NA
Lymphocyte count (×10^9/L)	0.63		1.90		NA		NA		1.6		NA
Monocyte count (×10^9/L)	0.16		1.4		NA		NA		0.7		NA
Neutrophil count (×10^9/L)	10.82		7.51		NA		NA		2.31		NA
Platelet count (×10^9/L)	267		280		NA		NA		211		NA
Red cell count (×10^9/L)	4.79		4.91		NA		NA		4.75		NA
Hypersensitive C-reactive protein (mg/L)	2.0		0.5		NA		NA		0.87		NA
ALT (U/L)	NA		10.1		12.7		NA		16.9		NA
AST (U/L)	NA		13.8		16.8		NA		16.8		NA
Albumin (g/L)	NA		40.9		40.8		NA		40.8		NA
Globin (g/L)	NA		26.7		24.8		NA		26.7		NA
Potassium (mmol/L)	NA		3.63		3.82		NA		3.82		NA
Sodium (mmol/L)	NA		139.0		139.00		NA		139		NA
Chloride (mmol/L	NA		101.00		108.00		NA		108.00		NA
Calcium (mmol/L)	NA		2.14		2.17		NA		2.14		NA
Fibrinogen (g/L)	NA		NA		3.29		NA		1.34		NA

Days post illness onset	Day 9				Day 16			Day 40			
Sample type	Blood	Urine	Throat swab	CSF	Blood	Urine	Throat swab	Blood	Urine	Throat swab	CSF
D-dimer (ng/ml)	NA	8.03		NA	NA			NA		NA	
Prothrombin activity (%)	NA	NA		73.40	NA			68.50		NA	
Cerebrospinal fluid											
Pressure	NA	195		130	125			130		140	
Appearance	NA	achromatic		achromatic	achromatic			achromatic		achromatic	
Transparency	NA	transparent		transparent	transparent			transparent		transparent	
Leukocyte count (×10^6/L)	NA	107		34	32			19		32	
Hyaline leukocyte	NA	92%		NA	NA			NA		NA	
Pleocaryocyte	NA	8%		NA	NA			NA		NA	
Glucose	NA	2.30		2.82	2.85			2.20		3.17	
Chloridion	NA	116.0		122.0	124.0			122.0		126	
Protein quantification	NA	988.50		654	654.00			475		414	

Clinical treatment	
Days 5–8 after illness onset	Antodin (2 mL intramuscularly × 1)
	Cefotaxime Sodium (3 g intravenously 2×/day on days 5 and 6)
	Levofloxacin (0.4 g intravenously 1×/day on day 8)
Day 9–21 after illness onset	Ceftriaxone sodium (1 g intravenously 2×/day on days 9–21)
	Invert sugarand electrolytes injection (500 mL given intravenously 2×/day on days 9–21)
	20% Mannitol injection (250 mL intravenously 2×/day on days 9 and 10)
	20% Mannitol injection (125 mL intravenously 3×/day on day 11)
	Esmerazole sodium (40 mg does intravenously 4×/day × on days 9 and 10)
	Aluminum phosphate gel (20 g intravenously 2×/day on days 9 and 10)
	20% Mannitol injection (125 mL intravenously 3×/day on days 15–17)
Day 33–45 after illness onset	Aciclovir (0.25 g intravenously 3×/day on days 33–45)
	Lansoprazole (30 mg intravenously 1×/day on days 33–43)
	Ringer sodium lactate (500 mL intravenously 1×/day on days 33–42)

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; CSF, cerebrospinal fluid; NA, not available because test was not performed or results were not reported; RT-PCR, reverse transcription-polymerase chain reaction; +, positive; –, negative.

Appendix Table 4. Primer sequences used to amplify the complete genome of Tacheng tick virus 2 isolated from a patient, China

Genome segment	Forward (location) (5′→ 3′)	Reverse (location) (5′→3′)
Large segment	LF1: AAGGGCACGCCACAACCC	LR511: GTTGGTCGTTGCCTTCCACG
	LF253: CTCACCCTTCAAGCCTCAACTC	LR992: TATGTGAAAGCCTCCCTCGCA
	LF812: CGGACCAGGACTACATCAAGAAG	LR1930: ATCCATCTGCTCCCTCCAACC
	LF1824: TCAGCAGGTGGGAGATTGGT	LR2743: ACACTGACGCAAGATGTGGTG
	LF2721: CACGCCCATGAACACACAT	LR3787: AACCACCTCCCTCAAGGACACT
	LF3725: CAATAGTGGCGAGGCAGGA	LR4624: GCTCGCATTGCCTCTTCTCT
	LF4376: GCACTTCTTGGATGGCACCT	LR5613: TTCTGCTGTCGTCCTCGTTG
	LF5289: GGGCACTGTCAACGGTCAAT	LR6546: GGCCCAACATCGTAATCCTCT
	LF6245: TAGAACAAAAGGAAGGCTTCTTCGAC	LR6632: CATAATCTCAAAGACCCTATACTGCCAC
Small segment	SF1: TGCCCCCCTCACTAAACAC	SR543: CTGGTGTTGATGCGTTGTCC
	SF344: CCTGCGTGGAAATTGATCTC	SR1597: ATCAGACGCACATCCATTCG
	SF1099: CTGGCAGAGTTCTCCAAGGTCA	SR1840: TTCCCTTGTGCTGCCATCCT
	SF1752: TAGCATTATGAGTGATGCCCAA	SR2185: TTCATTTAGTTTTACCTAGCTCCGAC

Appendix Table 5. Detection of Tacheng tick virus 2 by reverse transcription PCR in ticks collected from 9 counties and cities, Xinjiang Province, China

Tick species	County of sampling collection	Host	No. sampled	No. positive (%)
Dermacentor nuttalli	Qinghe	Sheep	86	7 (8.1)
	Wenquan	Free*	77	2 (2.6)
	Wusu	Free*	20	1 (5.0)
D. silvarum	Jinghe	Long-tailed ground squirrel	12	2 (16.7)
D. marginatus	Fuyun	Horse	44	10 (22.7)
	Gongliu	Sheep	32	2 (6.3)
	Xinyuan	Sheep	32	4 (12.5)
Hyalomma asiaticum	Shawan	Free*	21	1 (4.8)
	Fuhai	Camel	21	4 (19.0)
Total			345	33 (9.6)

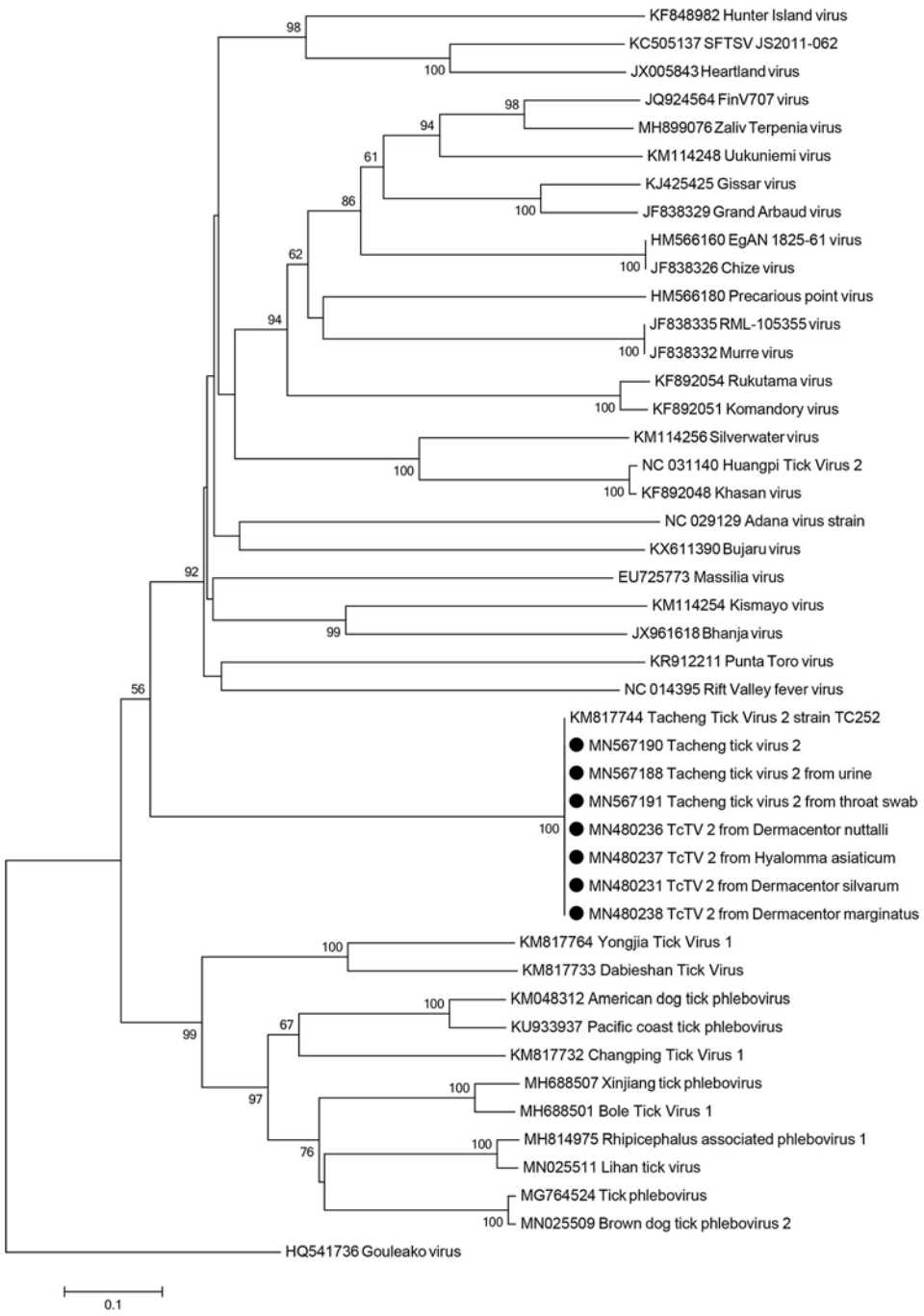
*Ticks collected from the sampling the environment.

Appendix Table 6. Primers used in a study of Tacheng tick virus 2 isolated from a human patient, China*

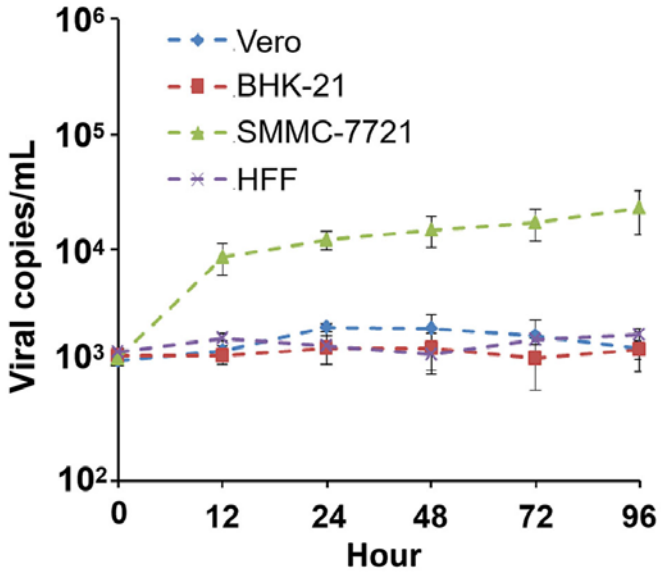
Primer name	Primer sequence (5′→3′)	Purification method
FinV707 virus M segment FW-1801	GATGTCATCTCATGGGAGCTTG	PAGE
FinV707 virus M segment DW-2240	CTCTTATTACAGACTGTATGGAA	PAGE
FinV707 virus M segment DW-2290	GTCGGCCAGAACGTGACTGAACC	PAGE
Zaliv virus M segment FW-1801	GGTGCCATTTGATGGGAGCTTG	PAGE
Zaliv virus M segment DW-2240	CTTCTATTGCAGACTGTATGGAA	PAGE
Zaliv virus M segment DW-2290	GTTGGCCAGAATGTGACAGAACC	PAGE
Uukuniemi virus M segment FW-1801	GATGCCATCTCATGGGAGCCTG	PAGE

Primer name	Primer sequence (5′→3′)	Purification method
Uukuniemi virus M segment DW-2240	CTTCTATTGCAGACAGTATGGAA	PAGE
Uukuniemi virus M segment DW-2290	GTTGGCCAGAAAGTGACTGAACC	PAGE
Chize-EgAN virus M segment-FW 1801	GGAGGTGCCATCTAATGGGAG	PAGE
Chize-EgAN virus M segment DW-2240	CTTCGATTGCAAACAGTATGGAA	PAGE
Chize-EgAN virus M segment DW-2290	CCAGAAGGTAACTGAGCCCAC	PAGE
Murre-RML-Precarious virus M segment FW-2040	TCTGAGCATTCAAACATGTTGTA	PAGE
Murre-RML virus M segment DW-2400	GATGGGTCAATAATGCTAGAT	PAGE
Precarious virus M segment DW-2400	GAAGGGTCAATGATGCTAGAT	PAGE
Murre-RML virus M segment DW-2610	CCCGGTTCACAATTATAACA	PAGE
Precarious virus M segment DW-2610	CCCGGTTCACAGTTATAACA	PAGE
Gissar-Grand arbaud virus M segment FW-270	GGCAGAAATGGATTAATCATGATGG	PAGE
Gissar-Grand arbaud virus M segment FW-550	AACTGGTTTTGGATTGATGG	PAGE
Gissar-Grand arbaud virus M segment DW-960	GAWCCTAGACATGCCCTGGTA	PAGE
Komandory-Rukutama virus M segment FW-30	ATGGAATCAACTATGAGAGGG	PAGE
Komandory-Rukutama virus M segment FW-1860	CTCGGAACAAGAAGATGCCATCTC	PAGE
Komandory-Rukutama virus M segment FW-2070	GTGTTTAACATGTTTGAATGT	PAGE
Komandory-Rukutama virus M segment DW-2230	CTCAGATTCTCCAAAGCATCTGTC	PAGE
Komandory-Rukutama virus M segment DW-2390	ATCATCAATATGGACTTTTGC	PAGE
Huangpi-Khasan virus M segment FW-1790	TGTCTAAAGAGTGATCTTTACTGG	PAGE
Khasan-Silverwater virus M segment FW-1720	TGTTGACTAGCCAGWCTGGTGA	PAGE
Khasan-Silverwater virus M segment DW-2310	CAYTGTATTTCTCCAAGCTT	PAGE
Huangpi-Khasan virus M segment DW-2260	TGCATGGTGCATGGAACATCTC	PAGE
Khasan-Huangpi Tick virus M segment FW-290	ATGGACTGTTCTGGTGGTAGG	PAGE
Silverwater virus M segment FW-280	GTGAGATGGACTGCTCTGGTGG	PAGE
Khasan-Huangpi Tick virus M segment FW-450	GATGACATGATCTGTCAGTTTGAG	PAGE
Silverwater virus M segment FW-450	GATGACATGATCTGCCAGTTCGAG	PAGE
Khasan-Huangpi Tick virus M segment DW-720	GAGCATCTCCTGTACATCTTCCTG	PAGE
Silverwater virus M segment DW-720	CCAGTGCATTTCCCAGCCTTACA	PAGE

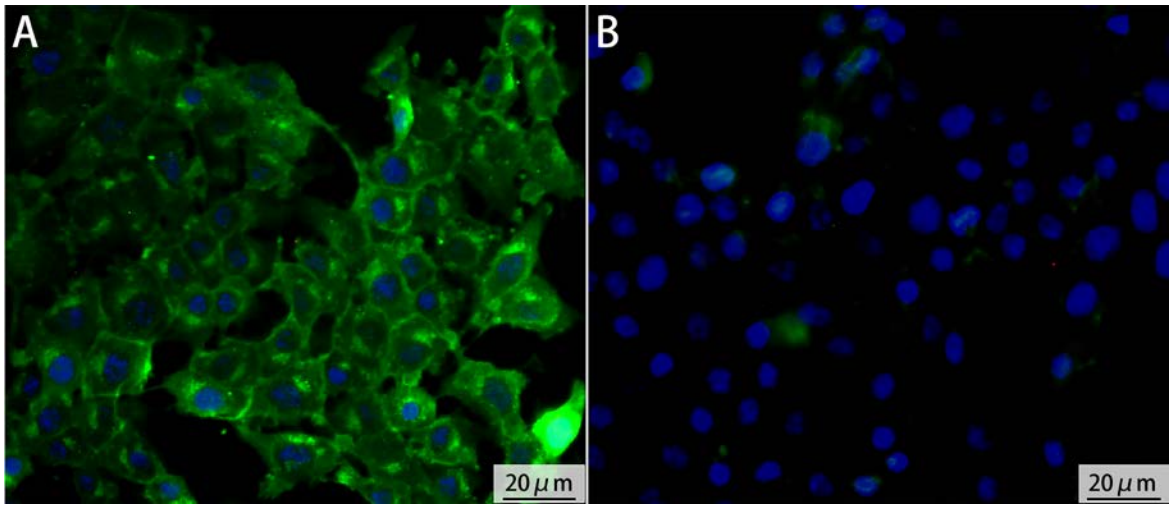
*We designed and synthesized a set of primers based on 15 tri-segment phleboviruses. In brief, we identified 14 tri-segment phleboviruses that cluster with Tacheng tick virus 2 and its related viruses based on phylogenetic analysis of the amino acid sequence for large segments by the neighbor-joining method. Viruses included Rukutama virus, Precarious point virus, RML-105355 virus, Murre virus, Komandory virus, Grand Arbaud virus, Silverwater virus, Huangpi tick virus 2, Khasan virus, Uukuniemi virus, Zaliv Terpenia virus, FinV707 virus, Chize virus, EgAN 1825–61 virus (Figure 2). We did not use Gissar virus because of its short sequence in GenBank. We then found common sequences through nucleotide sequence alignment function of 6 groups based on 15 trisegment phleboviruses by using DNAMAN software (Lynnon Biosoft Bioinformatic Solutions, https://www.lynnon.com). Finally, we used the selected common sequences to design primers. To enhance efficacy and possibility of reverse transcription PCR, we designed sets of seminested PCR primers for each virus group. M, medium.



Appendix Figure 1. Phylogenetic analysis based on partial amino acid sequences of the small segment of tickborne viruses. The tree was constructed using the neighbor-joining method by using MEGA version 7.0 (https://www.megasoftware.net). Black dots indicate Tacheng tick virus 2 isolated in this study (GenBank accession nos. MN567188, MN567190, MN567191, MN480231, MN480236, MN480237, and MN480238). Scale indicates amino acid substitutions per site.



Appendix Figure 2. Viral copies of Tacheng tick virus 2 over time in human hepatocellular carcinoma (SMMC-7721), African green monkey kidney (Vero), hamster kidney (BHK-21), and human foreskin fibroblast (HFF) cells. Estimates from the 2 technical replicates were averaged and error bars indicate standard deviation (SD) across biological replicates.



Appendix Figure 3. The detection of human hepatocellular carcinoma (SMMC-7721) cells infected with Tacheng tick virus 2 by immunofluorescence assay. A) The virus grown in SMMC-7721 cells detected by IFA; magnification × 200. B) SMMC-7721 cells without TcTV-2 infection, stained by the patient's convalescent serum; magnification × 200. IFA, immunofluorescence assay; TcTV-2, Tacheng tick virus 2.