

Human Tacheng Tick Virus 2 Infection, China, 2019

Zhihui Dong,¹ Meihua Yang,¹ Zedong Wang,¹ Shuo Zhao, Songsong Xie, Yicheng Yang, Gang Liu, Shanshan Zhao, Jing Xie, Quan Liu, Yuanzhi Wang

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 (BITV-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, BITV-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).

Author affiliations: Shihezi University, Shihezi, China (Z. Dong, M. Yang, Shuo Zhao, Y. Yang, G. Liu, Shanshan Zhao, Y. Wang); Foshan University, Foshan, China (Z. Wang, Q. Liu); First Affiliated Hospital of Shihezi University, Shihezi (S. Xie, J. Xie); Shihezi People's Hospital, Shihezi (Y. Yang)

DOI: <https://doi.org/10.3201/eid2702.191486>

¹These authors contributed equally to this article.

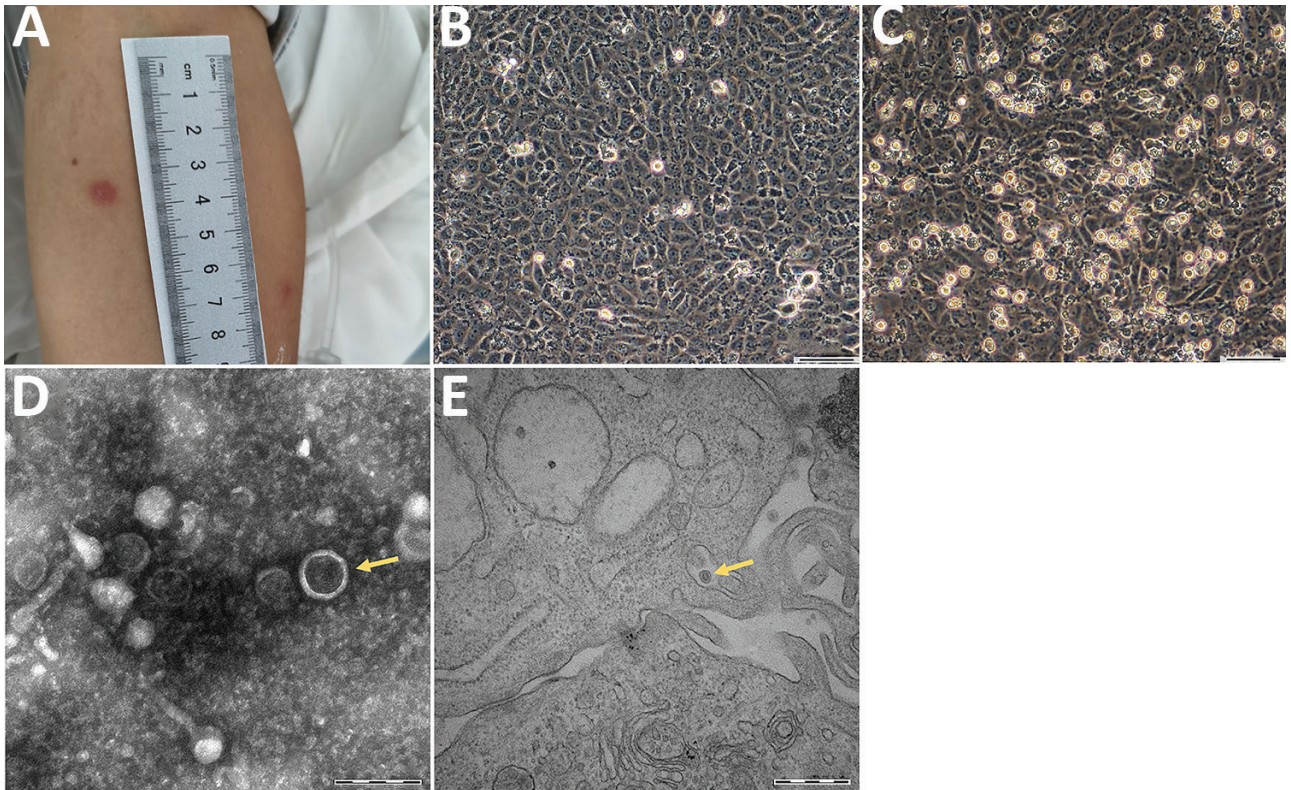


Figure 1. Clinical and morphological features of Tacheng tick virus 2 in a patient, China. A) Erythema at the site of tick bite on the anterior surface of the patient's left arm. B) Human hepatocellular carcinoma (SMMC-7721) cells without TcTV-2 infection; magnification $\times 100$. Scale bar indicates 50 μm . C) TcTV-2-infected SMMC-7721 cells showing cytopathic effects visible by light microscopy; magnification $\times 100$. Scale bar indicates 50 μm . D) Negatively stained virions purified from TcTV-2-infected SMMC-7721 cells (arrows); magnification $\times 25,000$. Scale bar indicates 200 nm. E) Transmission electron microscopy image of TcTV-2-infected SMMC-7721 cells (arrows); magnification $\times 50,000$. 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 $\approx 90\text{--}100$ 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).

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

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.

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, BITV-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

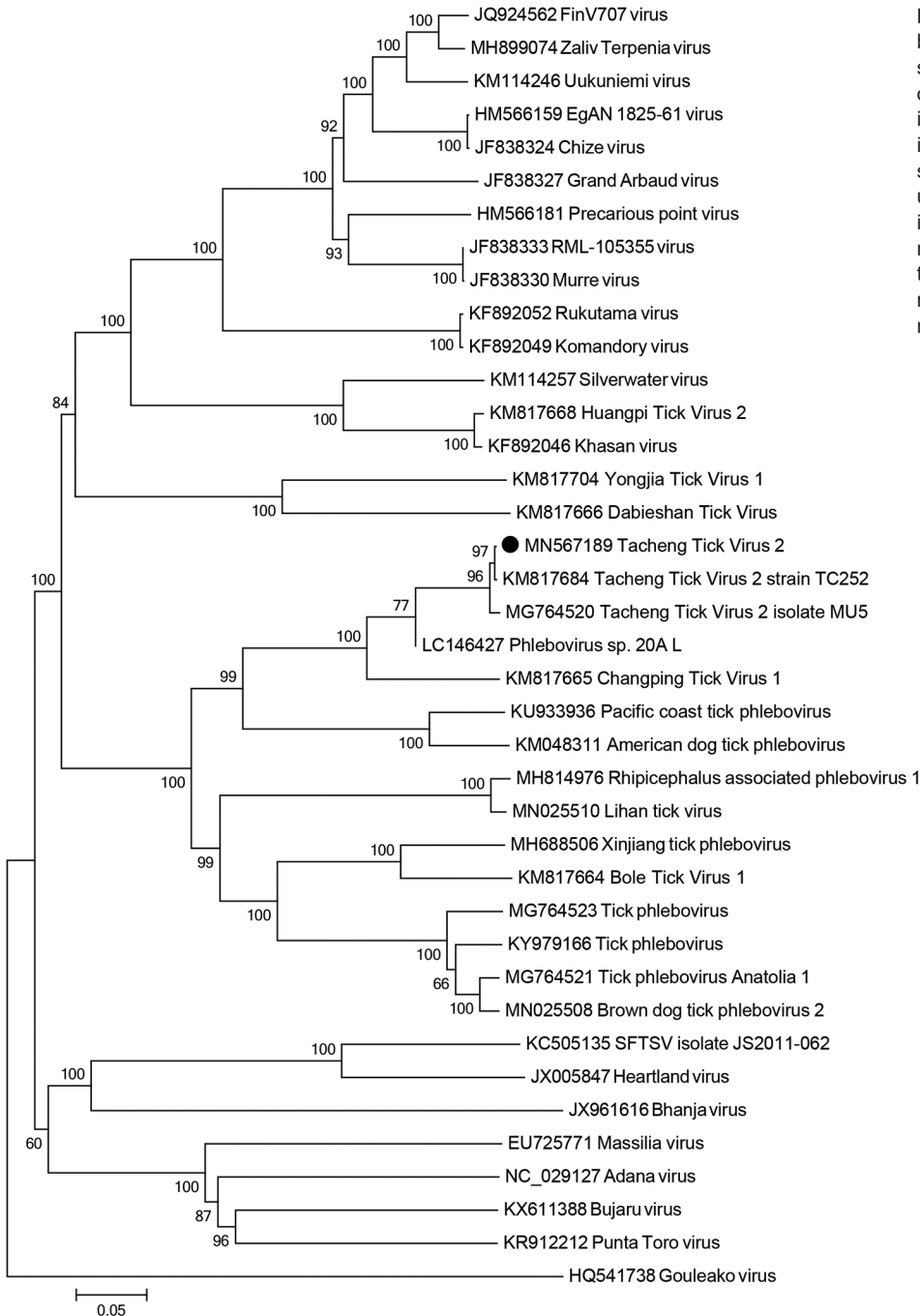


Figure 2. Phylogenetic analysis based on partial amino acid sequences of the L segment of tickborne viruses. Black dot indicates Tacheng tick virus 2 isolated from the patient in this study. The tree is constructed by using the neighbor-joining method in MEGA version 7.0 (<https://www.megasoftware.net>) 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.

This study was supported by the National Key Research and Development Program of China (approval no. 2018ZX10101002-002-007) and the National Natural Science Foundation of China (approval no. 81960379).

About the Author

Dr. Dong is a scientist at the School of Medicine, Shihezi University, Shihezi, China. Her research interest is emerging tickborne diseases.

References

1. Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med*. 2011;364:1523–32. <https://doi.org/10.1056/NEJMoa1010095>
2. Wang ZD, Wang B, Wei F, Han SZ, Zhang L, Yang ZT, et al. A new segmented virus associated with human febrile illness in China. *N Engl J Med*. 2019;380:2116–25. <https://doi.org/10.1056/NEJMoa1805068>
3. Jia N, Liu HB, Ni XB, Bell-Sakyi L, Zheng YC, Song JL, et al. Emergence of human infection with Jingmen tick virus in China: a retrospective study. *EBioMedicine*. 2019;43:317–24. <https://doi.org/10.1016/j.ebiom.2019.04.004>
4. Liu X, Zhang X, Wang Z, Dong Z, Xie S, Jiang M, et al. A tentative Tamdy Orthonairovirus related to febrile illness in northwestern China. *Clin Infect Dis*. 2020;70:2155–60. <https://doi.org/10.1093/cid/ciz602>
5. Li CX, Shi M, Tian JH, Lin XD, Kang YJ, Chen LJ, et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife*. 2015;4:e05378. <https://doi.org/10.7554/eLife.05378>
6. Zhang L, Li S, Huang SJ, Wang ZD, Wei F, Feng XM, et al. Isolation and genomic characterization of lymphocytic choriomeningitis virus in ticks from northeastern China. *Transbound Emerg Dis*. 2018;65:1733–9. <https://doi.org/10.1111/tbed.12946>
7. Tokarz R, Williams SH, Sameroff S, Sanchez Leon M, Jain K, Lipkin WI. Virome analysis of *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis* ticks reveals novel highly divergent vertebrate and invertebrate viruses. *J Virol*. 2014;88:11480–92. <https://doi.org/10.1128/JVI.01858-14>
8. Sameroff S, Tokarz R, Charles RA, Jain K, Oleynik A, Che X, et al. Viral diversity of tick species parasitizing cattle and dogs in Trinidad and Tobago. *Sci Rep*. 2019;9:10421. <https://doi.org/10.1038/s41598-019-46914-1>
9. Pereira A, Figueira L, Nunes M, Esteves A, Cotão AJ, Vieira ML, et al. Multiple Phlebovirus (Bunyaviridae) genetic groups detected in *Rhipicephalus*, *Hyalomma* and *Dermacentor* ticks from southern Portugal. *Ticks Tick Borne Dis*. 2017;8:45–52. <https://doi.org/10.1016/j.ttbdis.2016.09.015>
10. Dinçer E, Brinkmann A, Hekimoğlu O, Hacıoğlu S, Földes K, Karapınar Z, et al. Generic amplification and next generation sequencing reveal Crimean-Congo hemorrhagic fever virus AP92-like strain and distinct tick phleboviruses in Anatolia, Turkey. *Parasit Vectors*. 2017;10:335. <https://doi.org/10.1186/s13071-017-2279-1>

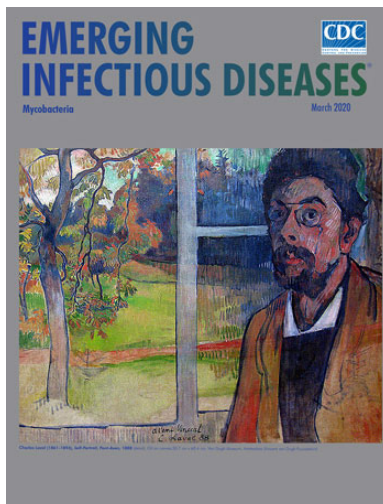
11. Souza WM, Fumagalli MJ, Torres Carrasco AO, Romeiro MF, Modha S, Seki MC, et al. Viral diversity of *Rhipicephalus microplus* parasitizing cattle in southern Brazil. *Sci Rep*. 2018;8:16315. <https://doi.org/10.1038/s41598-018-34630-1>
12. Brinkmann A, Dinçer E, Polat C, Hekimoğlu O, Hacıoğlu S, Földes K, et al. A metagenomic survey identifies Tamdy orthonairovirus as well as divergent phlebo-, rhabdo-, chu- and flavi-like viruses in Anatolia, Turkey. *Ticks Tick Borne Dis*. 2018;9:1173–83. <https://doi.org/10.1016/j.ttbdis.2018.04.017>
13. McMullan LK, Folk SM, Kelly AJ, MacNeil A, Goldsmith CS, Metcalfe MG, et al. A new phlebovirus associated with severe febrile illness in Missouri. *N Engl J Med*. 2012;367:834–41. <https://doi.org/10.1056/NEJMoa1203378>
14. Sheng J, Jiang M, Yang M, Bo X, Zhao S, Zhang Y, et al. Tick distribution in border regions of northwestern China. *Ticks Tick Borne Dis*. 2019;10:665–9. <https://doi.org/10.1016/j.ttbdis.2019.02.011>

Address for correspondence: Yuanzhi Wang or Quan Liu, Shihezi University, Shihezi, Xinjiang Uygur Autonomous Region 832002, China; email: wangyuanzhi621@126.com or liuquan1973@hotmail.com

March 2020

Mycobacteria

- Clinical Characteristics of Disseminated Strongyloidiasis, Japan, 1975–2017
- Epidemiology of Cryptosporidiosis, New York City, New York, USA, 1995–2018
- Public Health Response to Tuberculosis Outbreak among Persons Experiencing Homelessness, Minneapolis, Minnesota, USA, 2017–2018
- *Mycobacterium tuberculosis* Complex Lineage 3 as Causative Agent of Pulmonary Tuberculosis, Eastern Sudan
- Norovirus Outbreak Surveillance, China, 2016–2018
- Methicillin-Resistant *Staphylococcus aureus* Bloodstream Infections and Injection Drug Use, Tennessee, USA, 2015–2017
- Randomized Trial of 2 Schedules of Meningococcal B Vaccine in Adolescents and Young Adults, Canada
- Human Immune Responses to Melioidosis and Cross-Reactivity to Low-Virulence *Burkholderia* Species, Thailand
- Role of Live-Duck Movement Networks in Transmission of Avian Influenza, France, 2016–2017
- Multidrug- and Extensively Drug-Resistant *Mycobacterium tuberculosis* Beijing Clades, Ukraine, 2015
- Stable and Local Reservoirs of *Mycobacterium ulcerans* Inferred from the Nonrandom Distribution of Bacterial Genotypes, Benin
- Pulmonary *Nocardia ignorata* Infection in Gardener, Iran, 2017



- Long-Term Rodent Surveillance after Outbreak of Hantavirus Infection, Yosemite National Park, California, USA, 2012
- *Mycobacterium tuberculosis* Beijing Lineage and Risk for Tuberculosis in Child Household Contacts, Peru
- Risk Factors for Complicated Lymphadenitis Caused by Nontuberculous Mycobacteria in Children
- Human Exposure to Hantaviruses Associated with Rodents of the Murinae Subfamily, Madagascar
- Avian Influenza Virus Detection Rates in Poultry and Environment at Live Poultry Markets, Guangdong, China
- Diphtheria Outbreaks in Schools in Central Highland Districts, Vietnam, 2015–2018
- Progressive Vaccinia Acquired through Zoonotic Transmission in a Patient with HIV/AIDS, Colombia
- Suspected Locally Acquired Coccidioidomycosis in Human, Spokane, Washington, USA
- *Mycobacterium senegalense* Infection after Implant-Based Breast Reconstruction, Spain
- Low Prevalence of *Mycobacterium bovis* in Tuberculosis Patients, Ethiopia
- Metagenomics of Imported Multidrug-Resistant *Mycobacterium leprae*, Saudi Arabia, 2017
- Need for BCG Vaccination to Prevent TB in High-Incidence Countries and Populations
- US Tuberculosis Rates among Persons Born Outside the United States Compared with Rates in Their Countries of Birth, 2012–2016
- Genomic and Phenotypic Variability in *Neisseria gonorrhoeae* Antimicrobial Susceptibility, England
- Whole-Genome Sequencing to Detect Numerous *Campylobacter jejuni* Outbreaks and Match Patient Isolates to Sources, Denmark, 2015–2017
- Pregnancy Outcomes among Women Receiving rVSVΔ-ZEBOV-GP Ebola Vaccine during the Sierra Leone Trial to Introduce a Vaccine against Ebola [
- Acquisition of Plasmid with Carbapenem-Resistance Gene *bla*_{KPC2} in Hypervirulent *Klebsiella pneumoniae*, Singapore

**EMERGING
INFECTIOUS DISEASES**

To revisit the March 2020 issue, go to:
<https://wwwnc.cdc.gov/eid/articles/issue/26/3/table-of-contents>

Human Tacheng Tick Virus 2 Infection, China, 2019

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 | CTGGCTTGATTGGAAGGAC | | |
| 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 | CTCTGAGTGGGTTCTGATT | | |
| 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 | TCCTCACCCCTCAAGCCTCAACTCACTTTTTCCATGATTCACGTTCCAGTACC TGTCACGAGACACAGATGTTCCCTTCAAGAAATTCTATGAGACTGTGAATGAT GGCTTTGACAACCACACCCCTGATGTCATAATTGAAGACATCCAAGG | 152/153 (99) |
| 2 | 921–1101 | GGATCTCAATTGGCTAGAATGCACCAAGAAGGTAAATGAGTACAGGACTTCCT TCCACAAGCCTCGCAAAATGCGAGGGAGGCTTTCACATAAATCAACTATCCC CTTCCCAGGCTTTATGCCCATGGTATCTGAATATCCTTCCATTAGCGTCAAGG AGATCCTCAAAAAACCCTCCAACCTCCCCCA | 186/187 (99) |
| 3 | 1668–1788 | GGGGCCGGGTGAGTACATTATCAAGAAGCTGAGGAACTTTGAGGTCTTCCTG CTCATAAAGCCAACCAAGAGCAATGGGCCGATGTTTCGTATCTCTGGCTTGGC ATGCAAAAGATGTGTCTAACACATACCTTACAGACTACAT | 135/141 (96) |
| 4 | 1708–1828 | GTCTTCCTGCTCATAAAGCCAACCAAGAGCAATGGGCCGATGTTTCGTATCTCT GGCTTGGCATGCAAAAGATGTGTCCAACACATACCTTACAGACTACATGGTGT TCAAGGCCACTCAGCAGGTGGGAGACTGGTGTGACTGAATTCCATTCTT AAAACCATCCA | 163/169 (96) |
| 5 | 2088–2208 | CGGGCTTTGTGTCAGATCCCCTGTCTGCCCATCCTCACAAGATGATCGAAAA GCTTCCAG ATGTGGCCAGGACCAAACTCCAGGTCTGGCTGATCAACAAGAGCCTCAACCT CATGCAAG ACATTGCTCTGAGGCCTTATCAGCCA | 143/145 (99) |
| 6 | 2238–2558 | CCGCAGAATGCATTCAATACATCCATGTGCATTGATTGCACTATGTTGGGGCC CCAGGTTGCTTTGAGGTGCACCAGTGCCACGTACACACGTA CTTCTGAAAT GACACGGAGTACTCATGTGTCTTTATGTCGTCCAGAGGAGGATCCTTCCGGC CTA | 159/160 (99) |
| 7 | 4547–4667 | CCACATATATGTCCAAAGAGGGTCAAAACGTGCAGGGTCGAAGGAAGACAGT CCAAACCAGGGTGGTGGTTTTCCACAGAGAAGAGGCAATGCGAGCAAGGCC AGAAGATGTCCTCACAGATGTATGGTGGGGTCGA | 134/135 (99) |
| 8 | 4677–4857 | GGCGGGCCTCAGAAGGAGTGGCCTCACTTTGCCTGCACTGAAGGAGCATTTT GAGCAACTGCAGAGAGTGCTCCCATGGCTAACCAAGGATCCCAATGAGTCAT TGAGAAGATCCCCTTTCTTGCACCACCATCAACTCAGGAACCTTCTCTCGCGG 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 GGAAGGGTGCCGTGGCGCCCAAGGAGATCACCTGGCCAGGGTTGCCCTCACCT 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 | Throat | | CSF | Blood | Throat | | Blood | Throat | | CSF | |
| | | Urine | swab | | | Urine | swab | | Urine | swab | | |
| 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 ($\times 10^6/L$) | 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 \times 1) Cefotaxime Sodium (3 g intravenously 2 \times /day on days 5 and 6) Levofloxacin (0.4 g intravenously 1 \times /day on day 8) | | | | | | | | | | | |
| Day 9–21 after illness onset | Ceftriaxone sodium (1 g intravenously 2 \times /day on days 9–21) Invert sugarand electrolytes injection (500 mL given intravenously 2 \times /day on days 9–21) 20% Mannitol injection (250 mL intravenously 2 \times /day on days 9 and 10) 20% Mannitol injection (125 mL intravenously 3 \times /day on day 11) Esmerazole sodium (40 mg does intravenously 4 \times /day \times on days 9 and 10) Aluminum phosphate gel (20 g intravenously 2 \times /day on days 9 and 10) 20% Mannitol injection (125 mL intravenously 3 \times /day on days 15–17) | | | | | | | | | | | |
| Day 33–45 after illness onset | Aciclovir (0.25 g intravenously 3 \times /day on days 33–45) Lansoprazole (30 mg intravenously 1 \times /day on days 33–43) Ringer sodium lactate (500 mL intravenously 1 \times /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: CTCACCCCTCAAGCCTCAACTC | LR992: TATGTGAAAGCCTCCCTCGCA |
| | LF812: CGGACCAGGACTACATCAAGAAG | LR1930: ATCCATCTGCTCCCTCCAACC |
| | LF1824: TCAGCAGGTGGGAGATTGGT | LR2743: AACTGACGCAAGATGTGGTG |
| | LF2721: CACGCCCATGAACACACAT | LR3787: AACCACTCCCTCAAGGACACT |
| | LF3725: CAATAGTGGCGAGGCAGGA | LR4624: GCTCGCATTGCCTCTTCTCT |
| | LF4376: GCACTTCTTGGATGGCACCT | LR5613: TTCTGCTGTCGTCCTCGTTG |
| | LF5289: GGGCACTGTCAACGGTCAAT | LR6546: GGCCCAACATCGTAATCCTCT |
| | LF6245: TAGAACAAAAGGAAGGCTTCTTCGAC | LR6632: CATAATCTCAAAGACCCTATACTGCCAC |
| | Small segment | SF1: TGCCCCCTCACTAAACAC |
| SF344: CCTGCGTGGAATTGATCTC | | SR1597: ATCAGACGCACATCCATTCCG |
| 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 (%) |
|-----------------------------|-------------------------------|-----------------------------|-------------|------------------|
| <i>Dermacentor nuttalli</i> | Qinghe | Sheep | 86 | 7 (8.1) |
| | Wenquan | Free* | 77 | 2 (2.6) |
| | Wusu | Free* | 20 | 1 (5.0) |
| <i>D. silvarum</i> | Jinghe | Long-tailed ground squirrel | 12 | 2 (16.7) |
| <i>D. marginatus</i> | Fuyun | Horse | 44 | 10 (22.7) |
| | Gongliu | Sheep | 32 | 2 (6.3) |
| | Xinyuan | Sheep | 32 | 4 (12.5) |
| <i>Hyalomma asiaticum</i> | Shawan | Free* | 21 | 1 (4.8) |
| | Fuhai | Camel | 21 | 4 (19.0) |
| Total | | | 345 | 33 (9.6) |

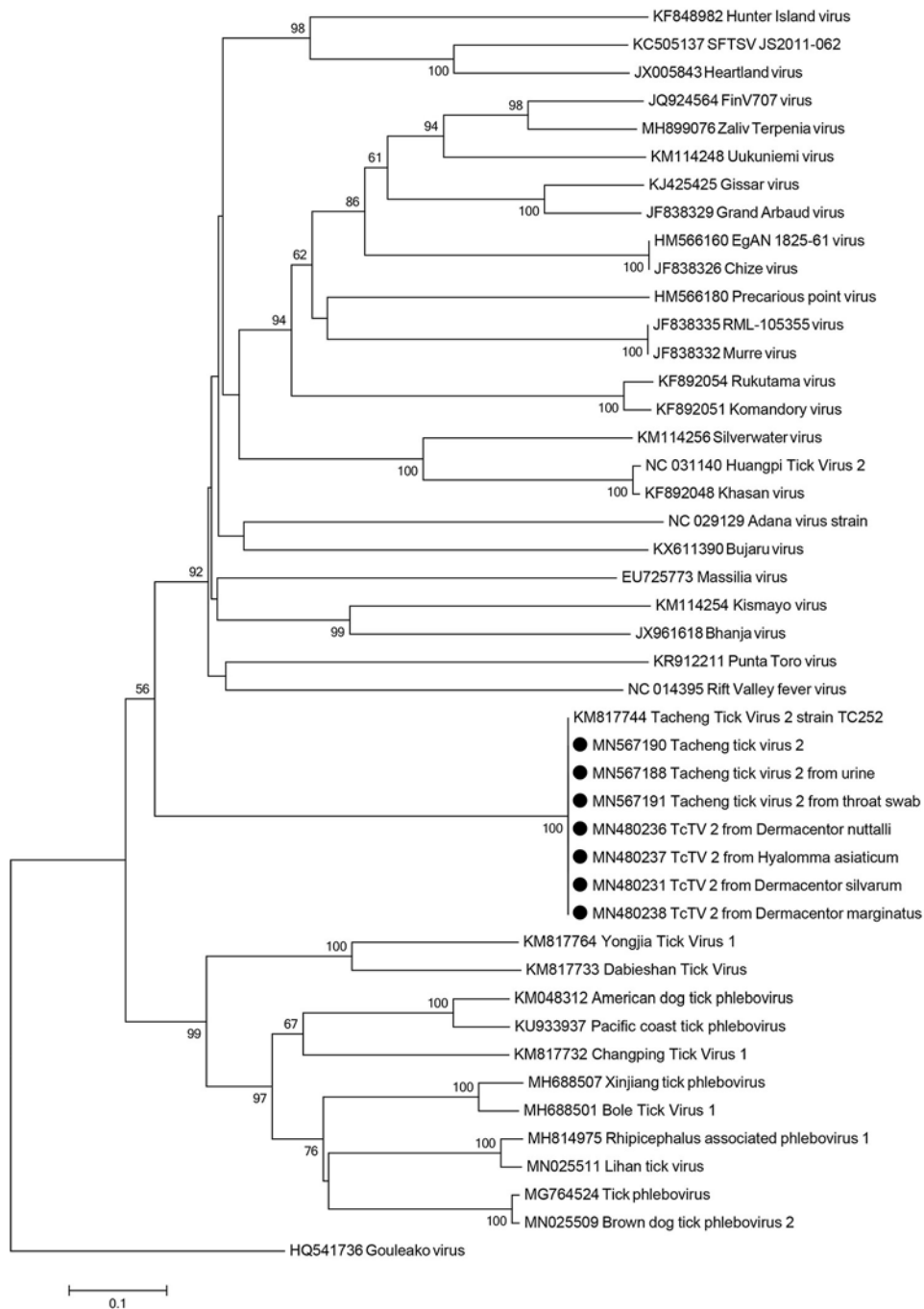
*Ticks collected from the sampling the environment.

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

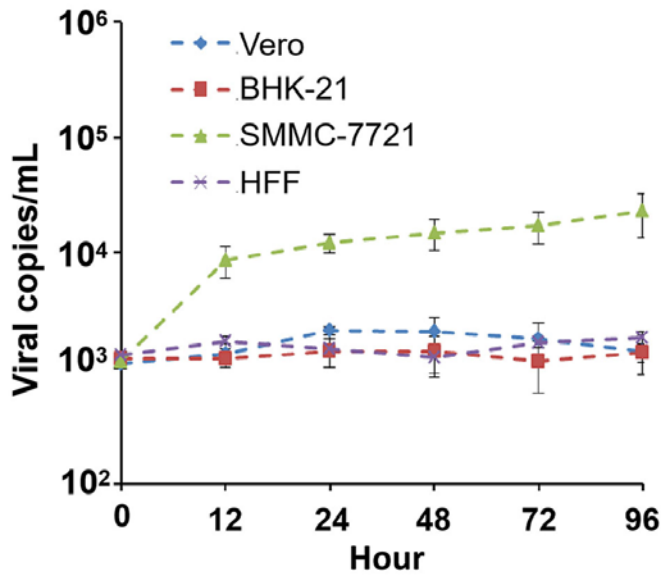
| Primer name | Primer sequence (5'→3') | Purification method |
|-----------------------------------|-------------------------|---------------------|
| FinV707 virus M segment FW-1801 | GATGTCATCTCATGGGAGCTTG | PAGE |
| FinV707 virus M segment DW-2240 | CTCTTATTACAGACTGTATGGAA | PAGE |
| FinV707 virus 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 | CCAGAAGGTAAGTACTGAGCCCAC | 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 | CCCGGTTTACAATTATAACA | PAGE |
| Precarious virus M segment DW-2610 | CCCGGTTTACAGTTATAACA | 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 | CTCAGATTCTCAAAGCATCTGTC | 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 | GATGACATGATCTGTCAGTTTGGAG | PAGE |
| Silverwater virus M segment FW-450 | GATGACATGATCTGCCAGTTCCGAG | PAGE |
| Khasan-Huangpi Tick virus M segment DW-720 | GAGCATCTCCTGTACATCTTCCTG | PAGE |
| Silverwater virus M segment DW-720 | CCAGTGCATTTCCCAGCCTTACA | PAGE |

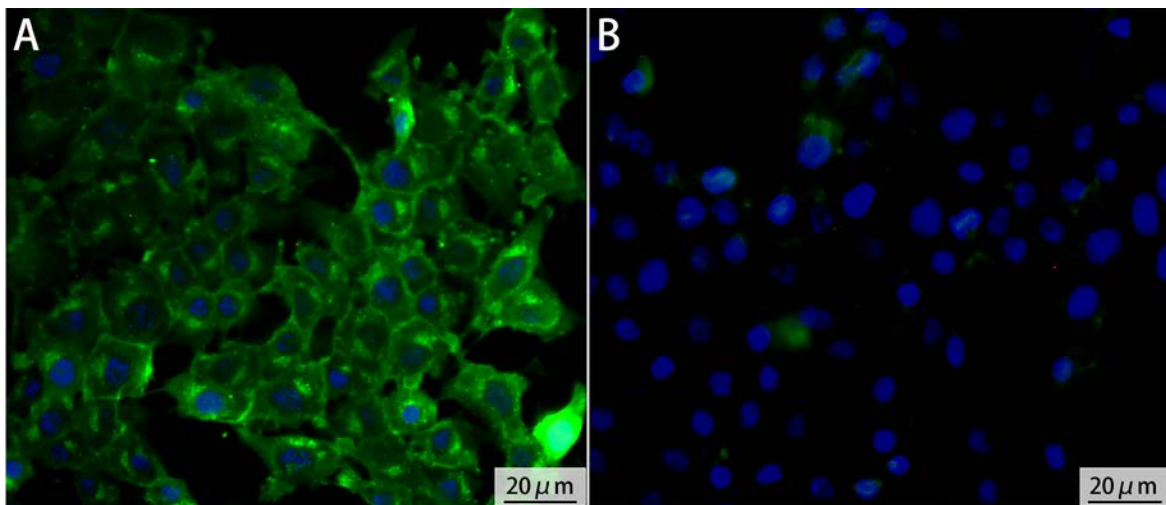
*We designed and synthesized a set of primers based on 15 tri-segment phleboviruses. In brief, we identified 14 tri-segment phleboviruses that cluster with Tacheng tick virus 2 and its related viruses based on phylogenetic analysis of the amino acid sequence for large segments by the neighbor-joining 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.