

Yezo Virus Infection in Tick-Bitten Patient and Ticks, Northeastern China

Xiaolong Lv,¹ Ziyang Liu,¹ Liang Li,¹ Wenbo Xu, Yongxu Yuan, Xiaojie Liang, Li Zhang, Zhengkai Wei, Liyan Sui, Yinghua Zhao, Zhijun Hou, Feng Wei, Shuzhen Han, Quan Liu, Zedong Wang

We identified Yezo virus infection in a febrile patient who had a tick bite in northeastern China, where 0.5% of *Ixodes persulcatus* ticks were positive for viral RNA. Clinicians should be aware of this potential health threat and include this emerging virus in the differential diagnosis for tick-bitten patients in this region.

Tickborne orthonairoviruses have been considered a major public health threat worldwide (1). In China, other than Crimean-Congo hemorrhagic fever virus, there are 3 emerging orthonairoviruses: Tacheng tick virus 1 (2), Songling virus (3), and Beiji nairovirus (4). Those viruses have been associated with human febrile illness in northeastern and northwestern China.

Yezo virus (YEZV), a new tickborne orthonairovirus discovered in Japan in 2021, can cause acute febrile illness in humans, whose clinical symptoms include thrombocytopenia and leukopenia (5). We report a case of YEZV infection in a tick-bitten patient and provide molecular evidence of YEZV infection in ticks in northeastern China.

The Study

The research protocol was approved by the human bioethics committee of Inner Mongolia General Forestry Hospital and the First Hospital of Jilin University, China. During 2018–2020, a total of 402 blood samples from tick-bitten patients were collected at the Inner Mongolia General Forestry Hospital (164 in 2018, 97 in 2019, and 141 in 2020) for viral detection by using reverse transcription PCR (Appendix Table 1

<https://wwwnc.cdc.gov/EID/article/29/4/22-0885-App1.pdf>). Results showed that 1 sample collected in 2018 was YEZV positive.

The patient was a 33-year-old man who lived on a farm in the Oroqen Autonomous Banner of Hulunbuir, Inner Mongolia, northeastern China, who had no history of underlying diseases. On June 18, 2018, he noticed a tick embedded on his back after he grazed horses on a mountain. The tick was removed intact by using tweezers in a local clinic and identified as *Ixodes persulcatus*. At that time, no obvious clinical symptoms, such as rash, itching, and discomfort, occurred. However, fever developed, followed by light headache, dizziness, blurred vision, chest distress, shortness of breath, fatigue, and arthralgia in 1 week (Table 1; Figure 1). No gastrointestinal (e.g., nausea, vomit, diarrhea) or hemorrhagic (e.g., melena, petechia, and ecchymosis) symptoms occurred.

Laboratory tests identified lymphocytopenia and neutrophilia, which accounted for 15.0% and 75.9% of the total leukocyte count, respectively. However, leukocyte counts (4,460 cells/mL) and platelet counts (199,000 cells/mL) were within reference ranges. Serum levels of liver aminotransferases were slightly increased (alanine aminotransferase 40 U/L, aspartate aminotransferase 44 U/L, and γ -glutamyltransferase 124 U/L) (Appendix Table 2). The serum level of C-reactive protein increased to 11.1 mg/L.

The patient was hospitalized for 8 days. Headache, dizziness, and arthralgia continued throughout the hospitalization, whereas the clinical signs of fever, blurred vision, chest distress, shortness of breath, and fatigue were relieved or disappeared (Figure 1). Lymphopenia continued until day 5, but counts of leukocytes, platelets, and erythrocytes and the hemoglobin level were all within reference ranges. Evidence of liver damage continued through the discharge date (alanine aminotransferase 56 U/L and γ -glutamyltransferase 181 U/L).

Author affiliations: First Hospital of Jilin University, Changchun, China (Z. Liu, W. Xu, L. Sui, Y. Zhao, Q. Liu, Z. Wang); Inner Mongolia General Forestry Hospital, Yakeshi, China (X. Lv, S. Han); Jilin Agricultural University, Changchun (Z. Liu, Y. Yuan, X. Liang, F. Wei); Chinese Academy of Agricultural Sciences, Changchun (L. Li, L. Zhang, Q. Liu, Z. Wang); Foshan University, Foshan, China (Z. Wei, Q. Liu); Northeast Forestry University, Harbin, China (Z. Hou)

DOI: <https://doi.org/10.3201/eid2904.220885>

¹These authors contributed equally to this article.

Table 1. Characteristics of a tick-bitten patient infected with Yezo virus, northeastern China*

Characteristic	Result and treatment
Fever	
Temperature at admission, °C	39.5
Highest temperature, °C	39.6
Complications	
Headache	Yes
Dizziness	Yes
Blurred vision	Yes
Arthralgia	Yes
Chest distress	Yes
Shortness of breath	Yes
Fatigue	Yes
Bacterial co-infection	No
Tickborne pathogen detection	
TBEV	Negative
SFTSV	Negative
Alongshan virus	Negative
Songling virus	Negative
Beiji nairovirus	Negative
Yezo virus	Positive
<i>Borrelia</i> spp.	Negative
<i>Rickettsia</i> spp.	Negative
<i>Anaplasma</i> spp.	Negative
<i>Babesia</i> spp.	Negative
Treatment	
Day 1–4	Ribavirin (1 g IV), azithromycin (0.5 g IV), rocephin (3 g IV) daily
Day 1–8	Glycyrrhizin (3 tablets orally daily)

*IV, intravenous; SFTSV, severe fever with thrombocytopenia syndrome virus; TBEV, tick-borne encephalitis virus.

A blood sample was collected at admission and was negative for the tick-borne pathogens that have been identified in northeastern China (Table 1). The patient was empirically given ribavirin (1 g), azithromycin (0.5 g), and rocephin (3 g) intravenously each day during the first 4 days of hospitalization (Table 1). Glycyrrhizin was used to treat liver injury. The patient was discharged on the 8th day of hospitalization, although some clinical manifestations, such as headache, dizziness, and arthralgia, were still present. Two weeks later, the patient had recovered completely.

During April 2020–July 2021, a total of 2,830 ticks were collected from Heilongjiang, Jilin, and

Inner Mongolia in northeastern China (214 *Haemaphysalis japonica*, 431 *H. concinna*, 1,110 *Dermacentor silvarum*, and 1,075 *I. persulcatus*) (Table 2). YEZV RNA was detected in *I. persulcatus* ticks by using reverse transcription PCR; overall prevalence was 0.5% (95% CI 0.2%–1.0%) (Table 2). The prevalence of YEZV infection in *I. persulcatus* ticks in Inner Mongolia, Heilongjiang, and Jilin varied from 0.4% to 0.5%. No positive sample was detected in other tick species.

We obtained complete genomes of 6 YEZV strains (1 from the patient and 5 from *I. persulcatus* ticks) by using specific primers (Appendix Table 1). YEZV has a genomic structure of typical orthonairoviruses (5). The complete genome of YEZV identified in this study included large (12,122-nt), medium (4,256-nt) and small (1,697-nt) segments (Appendix Table 3), which encoded a 3,938-aa large protein, a 1,356-aa glycoprotein precursor, and a 502-aa nucleocapsid (Appendix Figure 1). YEZV strain H-IM01 from the patient showed high sequence identities with those detected in ticks (T-HLJ01–03, T-JL01, and T-IM01) and nucleotide identities of 99.6%–100% (Appendix Tables 4, 5). Strains isolated from northeastern China were clustered with strains detected in tick-bitten patients in Japan and showed high nucleotide identities of 97.2%–98.8% (Appendix Tables 4, 5).

The YEZV strains from China were genetically related to Sulina virus discovered in *I. ricinus* ticks in Romania (6), showing complete genome nucleotide identities of 59.7%–70.3% and large protein amino acid identities of 82.3%–82.5%; they were grouped into the genogroup Sulina (Figure 2; Appendix Tables 4, 5, Figure 2) (5). Phylogenetic analysis indicated that viruses in the genogroup Sulina had a close relationship with Tamdy virus; those viruses showed nucleotide identities of ≈50% and large protein amino acid identities of ≈45% with each other. All viral genome sequences have been submitted to GenBank (Appendix Table 3).

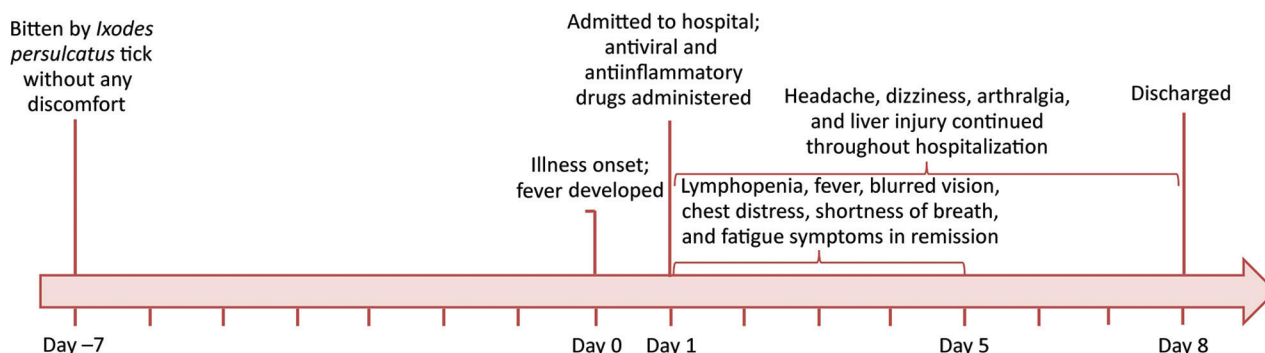
**Figure 1.** Timeline of the clinical course for a tick-bitten person infected with Yezo virus, northeastern China.

Table 2. Detection of Yezo virus RNA in *Ixodes persulcatus* ticks, northeastern China*

Province	Sampling time	No. pools/no. ticks	No. positive pools	Positive rate (95% CI)
Heilongjiang	2021	63/625	3	0.5 (0.1–1.3)
Jilin	2020–2021	19/192	1	0.5 (0.0–2.5)
Inner Mongolia	2021	26/258	1	0.4 (0.0–1.9)
Total	2020–2021	108/1,075	5	0.5 (0.2–1.0)

*Prevalence of Yezo virus infection in ticks was calculated by using PooledInfRate Excel Add-In version 4.0 (Microsoft, <https://www.microsoft.com>) and a 1-sample analysis with a bias-corrected maximum likelihood estimation method with 95% CIs and a scale of 100.

Conclusions

Clinical manifestations of the YEZV-infected patient in China were milder than those reported for patients in Japan, where leukopenia, lymphocy-

topenia, thrombocytopenia, coagulation disorder, and increased levels of liver and heart enzymes have been observed (5); only mild lymphocytopenia and mildly increased levels of liver enzymes were

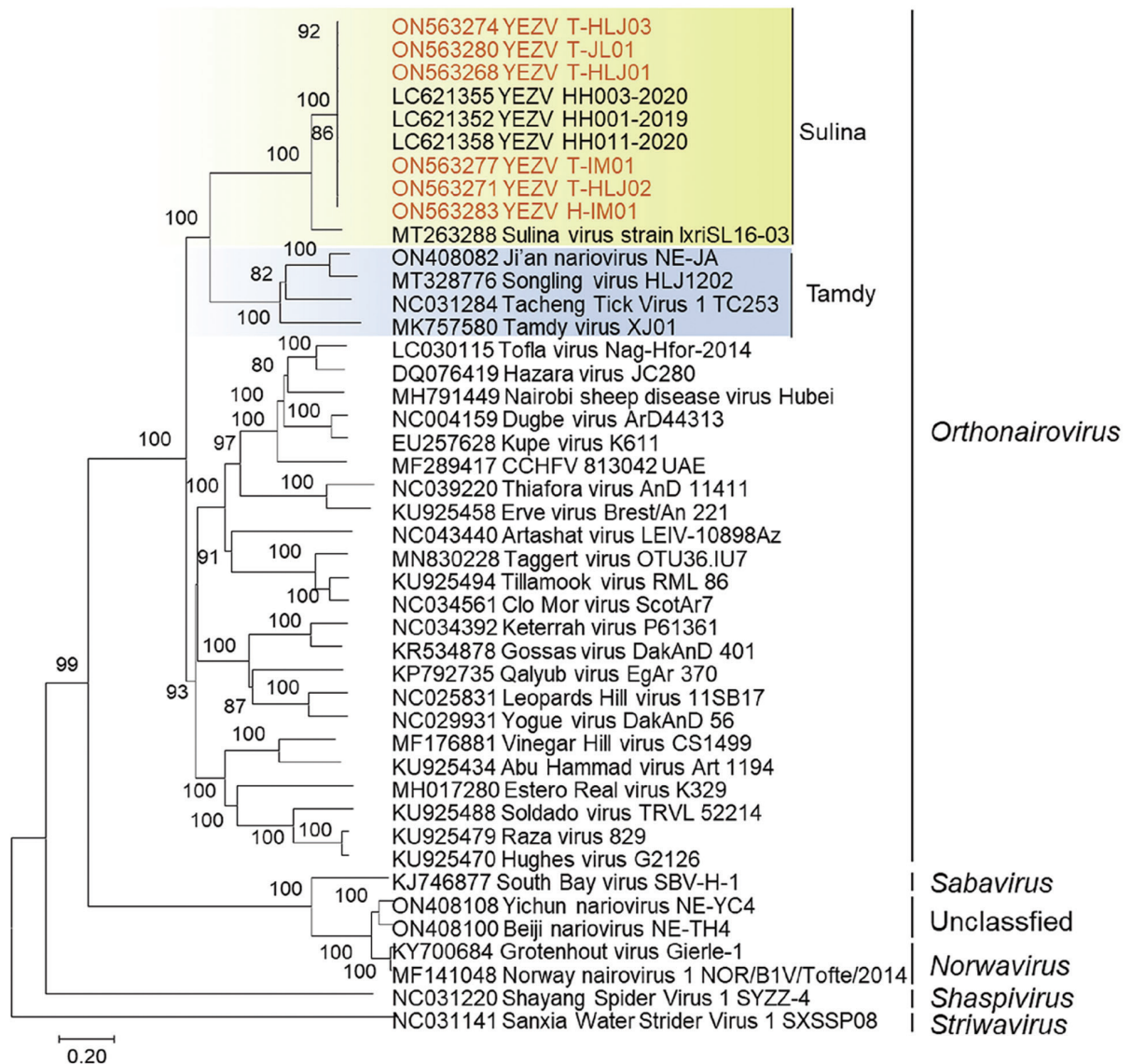


Figure 2. Phylogenetic analyses of Yezo virus from a tick-bitten person and *Ixodes persulcatus* ticks, northeastern China (red text), and references viruses. Sequences of representative viral strains were downloaded from National Center for Biotechnology Information public databases (<https://www.ncbi.nlm.nih.gov>) and aligned together using MEGA version 7.0 (<https://www.megasoftware.net>). A bootstrapping analysis of 1,000 replicates were conducted, and values >70 were considered significant and are shown. Shading indicates Sulina virus genogroup strains (green) and Tamdy virus strain (blue). Numbers along branches are bootstrap values. Scale bar indicates amino acid substitutions per site.

found in the patient in this study. The 2 patients in Japan were a 59-year-old man and a 41-year-old man who had medical histories of hyperuricemia and hyperlipidemia, and the patient from China was a 33-year-old man who had no underlying disease. The YEZV-infected patient in this study was given ribavirin on days 1–4, but the patients in Japan were not given ribavirin. The clinical signs of YEZV infection might be related to age, medical history, and medication. No gastrointestinal symptoms, such as nausea, vomiting, and diarrhea, or hemorrhagic symptoms, such as melena, petechia, and ecchymosis, occurred in any of the YEZV-infected patients.

The infection rate of YEZV was low (1/402) in tick-bitten patients in northeastern China, compared with YEZV patients in Japan (5/248). To date, no severe YEZV-infected patient has been reported, and the patients in the 2 countries recovered completely. Thus, active surveillance should be performed on the tick-bitten populations to evaluate the prevalence and clinical characteristics of YEZV infection in the studied regions.

YEZV RNA has been detected in *H. megaspinosus*, *I. ovatus*, and *I. persulcatus* ticks in Hokkaido, Japan, showing a prevalence of 0.0%–5.7% (5). In this study, YEZV was detected only in *I. persulcatus* ticks, showing a prevalence of 0.5% (95% CI 0.2%–1.0%), and other tick species, such as *H. japonica*, *H. concinna*, and *D. silvarum*, were negative for YEZV RNA. Those results indicate that *I. persulcatus* ticks might serve as a potential vector for YEZV in northeastern China.

YEZV was identified in a tick-bitten patient who had febrile illness and *I. persulcatus* tick bites in northeastern China. Phylogenetic analysis confirmed the association between febrile illness and the virus. To date, there are ≥8 pathogenetic tickborne viruses in humans and animals found in northeastern China: tickborne encephalitis virus (7), severe fever with thrombocytopenia syndrome virus (8), Nairobi sheep disease virus (9), Alongshan virus (10), Jingmen tick virus (11), Songling virus (3), Beiji nairovirus (4), and YEZV. Differential diagnosis of these tickborne viruses should be conducted in febrile patients who have a history of tick bites in northeastern China.

This study was supported by the National Key Research and Development Program of China (2022YFC2601900), the National Natural Science Foundation of China (82002165 and 32072887), the Outstanding Young Scholars Cultivating Plan of the First Hospital of Jilin University (2021-YQ-01), the Medical and Health Talent Special Project in Jilin Province of China (JLSWSRCZX2021-002), the Pearl River Talent Plan in Guangdong Province of China

(2019CX01N111), and the Medical Innovation Team Project of Jilin University (2022JBGS02).

About the Author

Mr. Lv is a scientist at the Second Affiliated Hospital of Inner Mongolia University for the Nationalities, Inner Mongolia General Forestry Hospital, Yakeshi, China. His primary research interest is emerging tickborne diseases.

References

- Walker PJ, Widen SG, Wood TG, Guzman H, Tesh RB, Vasilakis N. A global genomic characterization of nairoviruses identifies nine discrete genogroups with distinctive structural characteristics and host-vector associations. *Am J Trop Med Hyg*. 2016;94:1107–22. <https://doi.org/10.4269/ajtmh.15-0917>
- 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>
- Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat Med*. 2021;27:434–9. <https://doi.org/10.1038/s41591-020-01228-y>
- Wang YC, Wei Z, Lv X, Han S, Wang Z, Fan C, et al. A new nairo-like virus associated with human febrile illness in China. *Emerg Microbes Infect*. 2021;10:1200–8. <https://doi.org/10.1080/22221751.2021.1936197>
- Kodama F, Yamaguchi H, Park E, Tatamoto K, Sashika M, Nakao R, et al. A novel nairovirus associated with acute febrile illness in Hokkaido, Japan. *Nat Commun*. 2021;12:5539. <https://doi.org/10.1038/s41467-021-25857-0>
- Tomazatos A, von Possel R, Pekarek N, Holm T, Rieger T, Baum H, et al. Discovery and genetic characterization of a novel orthonairovirus in *Ixodes ricinus* ticks from Danube Delta. *Infect Genet Evol*. 2021;88:104704. <https://doi.org/10.1016/j.meegid.2021.104704>
- Sun RX, Lai SJ, Yang Y, Li XL, Liu K, Yao HW, et al. Mapping the distribution of tick-borne encephalitis in mainland China. *Ticks Tick Borne Dis*. 2017;8:631–9. <https://doi.org/10.1016/j.ttbdis.2017.04.009>
- Zhang X, Wang N, Wang Z, Che L, Chen C, Zhao WZ, et al. First fatal infection and phylodynamic analysis of severe fever with thrombocytopenia syndrome virus in Jilin Province, northeastern China. *Virology*. 2021;36:329–32. <https://doi.org/10.1007/s12250-020-00228-z>
- Gong S, He B, Wang Z, Shang L, Wei F, Liu Q, et al. Nairobi sheep disease virus RNA in ixodid ticks, China, 2013. *Emerg Infect Dis*. 2015;21:718–20. <https://doi.org/10.3201/eid2104.141602>
- 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>
- 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>

Address for correspondence: Zedong Wang, Department of Infectious Diseases, Center of Infectious Diseases and Pathogen Biology, State Key Laboratory of Zoonotic Diseases, First Hospital of Jilin University, Changchun 130122, Jilin, China; email: wangzedong@jlu.edu.cn

EID cannot ensure accessibility for supplementary materials supplied by authors. Readers who have difficulty accessing supplementary content should contact the authors for assistance.

Yezo Virus Infection in Tick-Bitten Patient and Ticks, Northeastern China

Appendix

Appendix Table 1. Primers used in this study

Segment	Primer	Position (bp)*	Sequence (5'→3')	Polarity	Amplicon (bp)
Detection					
	YEZV F	1412	ACCCACATACTCTCTAYAACGA	+	255
	YEZV R2	1646	TACTGTGGCCTTGATCTCACT	-	
	YEZV R1	1679	CTGAAGCCTCCCTAAGTGC	-	
Genome amplification					
Large	1 YEZV F	1	ACATCTATCCTGCAATCCC	+	964
	1 YEZV R1	962	GGTGYCTYCCAACCTTTCCC	-	
	1 YEZV R2	937	TGTTTGTTTCGTTTACCAGC	-	
	2 YEZV F1	869	CTACTGTCCAAGCCAACCA	+	1218
	2 YEZV F2	900	GTCCTGTTTCGTAGACTCCCAGC	+	
	2 YEZV R	2099	TCAAAMAGAACCTCGCACTC	-	
	3 YEZV F1	2017	AGTGCCATAGCAATYATC	+	1066
	3 YEZV R2	3063	TCTGTCCATTGTAACYCCTT	-	
	3 YEZV R1	3272	CTGGCTATACCTTTGATTGCAT	-	
	4 YEZV F	2874	TCATGGTTGCCAACATCAGC	+	1029
	4 YEZV R2	3903	ATGAAGTTTAACTCTTTTCGC	-	
	4 YEZV R1	3976	GTCAGACTTGCCACTATA	-	
	5 YEZV F	3751	AGCCTGCAAGATAATGAACAC	+	926
	5 YEZV R2	4658	CCAGCCCAAACACAACCTGA	-	
	5 YEZV R1	4836	ATACCAGCTGTAAGTAAAGCC	-	
	6 YEZV F	4597	GACAGGCAACTTTCTTGACC	+	1028
	6 YEZV R1	5786	TTTCTGTGCCTCTGTTAGTGT	-	
	6 YEZV R2	5605	TTGGCTCAGACACAATGGAT	-	
	7 YEZV F	5493	TCATTCAAGCYTTCACGGAG	+	1147
	7 YEZV R1	6684	CTCATCAGGCTTCATTGCAT	-	

Segment	Primer	Position (bp)*	Sequence (5'→3')	Polarity	Amplicon (bp)
	7 YEZV R2	6619	CAGCAGTATCTGTATAGCCAA	-	
	8 YEZV F	6500	GCAWTGAAACGGCTTACTTC	+	1098
	8 YEZV R1	7764	TCTTACTTATTGCTCCGCTGA	-	
	8 YEZV R2	7577	GTTGTAACATGTCATCGCCAT	-	
	9 YEZV F1	7446	TGGCAAGCAAATACAAACTCG	+	1156
	9 YEZV F2	7526	GACCAGCCCATGATACAGT	+	
	9 YEZV R	8661	ATCTAACAAGGCCAAATGTCC	-	
	10 YEZV F	8559	AGGATTATGACGGATTCCCT	+	946
	10 YEZV R1	9577	GGCCTCCGTAATCAACCCTG	-	
	10 YEZV R2	9485	TTGACCTGCTCAGATGCATT	-	
	11 YEZV F	9377	ATGCAGTTTTTCAGAGCCGAA	+	1174
	11 YEZV R1	10622	GCGGTTCACTACAGTCACA	-	
	11 YEZV R2	10531	GTGTAAAGCCCAACATCCTT	-	
	12 YEZV F	10450	CCTGCTTCCAACCTATCCAA	+	913
	12 YEZV R1	11371	AGACCTCTGTCATCTAGGCTR	-	
	12 YEZV R2	11344	CAGTTCGTTCTTGCACTC	-	
	13 YEZV F1	10901	GAGCTAAGCAGCAAGTCACA	+	985
	13 YEZV F2	11137	CAGCAACATTGACGTCCARC	+	
	13 YEZV R	12101	GTTCTGCATACCCCCCTATTA	-	
Medium					
	1 YEZV F	1	ACTTGCAGCGACCCCCCAAAG	+	832
	1 YEZV R1	851	TCCACCACTGCCTTACAGCC	-	
	1 YEZV R2	813	ATAACCAGTWAGATAGGCAA	-	
	2 YEZV F	763	ATCAGARAATATAAAGGTCAGC	+	835
	2 YEZV R1	1631	GCTCGACAGACATAGATTGC	-	
	2 YEZV R2	1576	TAGATCAACCTTATTGGTGTGC	-	
	3 YEZV F	1518	CTGCTCCTAAAGGAAGTCTWG	+	836
	3 YEZV R1	2397	CCCTATTTTGACTTTGCCAAC	-	
	3 YEZV R2	2333	CTCAATATGAACAGTACCCCA	-	
	4 YEZV F1	2217	GYCCAATTCAAGAATCRCCTA	+	834
	4 YEZV R	3094	ATCTACTAAGCCATTGAGGTTG	-	
	4 YEZV F2	2282	CTTCCCTRCCCGTAGCWG	+	
	5 YEZV F1	2893	ATCTCTAGGAGGCATATCAGT	+	763
	5 YEZV F2	2956	CCAGATCGCTCTAGTACACA	+	
	5 YEZV R	3699	CTCYGACTCTTTTGGTGCTT	-	
	6 YEZV F1	3535	AACATTRGACAGTCCYGACCGG	+	649
	6 YEZV F2	3607	CACYCTAACTTGACGCGCT	+	
	6 YEZV R	4237	AGTGGCAGCATACCCCCCTT	-	

Segment	Primer	Position (bp)*	Sequence (5'→3')	Polarity	Amplicon (bp)
Small					
	1 YEZV F	1	CGTGCTGCGACCCCCCAATAG	+	854
	1 YEZV R2	766	CTTCTTCTGTACAAGGTGC	-	
	1 YEZV R1	840	TCCATCTTCTGCTGGTTGT	-	
	2 YEZV F1	607	GCAGGAACAAGAAGCACTC	+	608
	2 YEZV R	1273	AGCCTTCTTTGATCTTRACA	-	
	2 YEZV F2	684	ATAGGATCTTYAGCCTGCCTTC	+	464
	3 YEZV F1	1152	CYAACATGTGTGCAACRGA	+	
	3 YEZV R	1676	CCTAGAAGCGGGAAGATGTTGT	-	
	3 YEZV F2	1233	AGATGACTGCWGCCATTGGC	+	

*The position of primers referred to the complete sequences obtained from RNA-seq in this study.

Appendix Table 2. Clinical blood biochemistry test results of the patient at admission

Test	Result	Normal range
Leukocytes count ($\times 10^9$ per L)	4.46	4.0–10.0
Lymphocyte cells count ($\times 10^9$ per L)	0.67	0.90–4.52
Monocytes count ($\times 10^9$ per L)	0.39	0.1–0.6
Neutrophils count ($\times 10^9$ per L)	3.38	1.55–6.27
Eosinophils count ($\times 10^9$ per L)	0.01	0.0–0.76
Basophils count ($\times 10^9$ per L)	0.0	0.0–0.1
Lymphocyte percentage (%)	15.0	22.6–49.4
Monocytes percentage (%)	8.7	3.0–10.0
Neutrophils percentage (%)	75.9	39.0–68.5
Eosinophils percentage (%)	0.2	0.4–8.0
Basophils percentage (%)	0.0	0.0–1.0
Red cells count ($\times 10^{12}$ per L)	5.02	4.30–5.80
Concentration of hemoglobin (g/L)	158.0	131.0–172.0
Platelet count ($\times 10^9$ per L)	199	125–350
C-reactive protein (mg/l)	11.1	0.0–3.0
Alanine aminotransferase (U/L)	40	9–50
Aspartate aminotransferase (U/L)	44	15–40
Alkaline phosphatase (U/L)	45	45–125
γ -glutamyltransferase	124	8–57

*Abnormal values are indicated in bold.

Appendix Table 3. Nucleotide sequence information of the Yezo virus identified in the present study

Strain	Place of sampling in China	Host species	Gene	GenBank Accession no.
T-HLJ01	Heilongjiang	<i>Ixodes persulcatus</i>	L	ON563268
T-HLJ01	Heilongjiang	<i>Ixodes persulcatus</i>	M	ON563269
T-HLJ01	Heilongjiang	<i>Ixodes persulcatus</i>	S	ON563270
T-HLJ02	Heilongjiang	<i>Ixodes persulcatus</i>	L	ON563271
T-HLJ02	Heilongjiang	<i>Ixodes persulcatus</i>	M	ON563272
T-HLJ02	Heilongjiang	<i>Ixodes persulcatus</i>	S	ON563273
T-HLJ03	Heilongjiang	<i>Ixodes persulcatus</i>	L	ON563274
T-HLJ03	Heilongjiang	<i>Ixodes persulcatus</i>	M	ON563275
T-HLJ03	Heilongjiang	<i>Ixodes persulcatus</i>	S	ON563276
T-IM01	Hulunbuir, Inner Mongolia	<i>Ixodes persulcatus</i>	L	ON563277
T-IM01	Hulunbuir, Inner Mongolia	<i>Ixodes persulcatus</i>	M	ON563278
T-IM01	Hulunbuir, Inner Mongolia	<i>Ixodes persulcatus</i>	S	ON563279
T-JL01	Jilin	<i>Ixodes persulcatus</i>	L	ON563280
T-JL01	Jilin	<i>Ixodes persulcatus</i>	M	ON563281
T-JL01	Jilin	<i>Ixodes persulcatus</i>	S	ON563282
H-IM01	Hulunbuir, Inner Mongolia	Human	L	ON563283
H-IM01	Hulunbuir, Inner Mongolia	Human	M	ON563284
H-IM01	Hulunbuir, Inner Mongolia	Human	S	ON563285

Appendix Table 4. Nucleotide sequence identities of large segment (lower left) and amino acid sequence identities of RdRp (upper right) of YEZV*

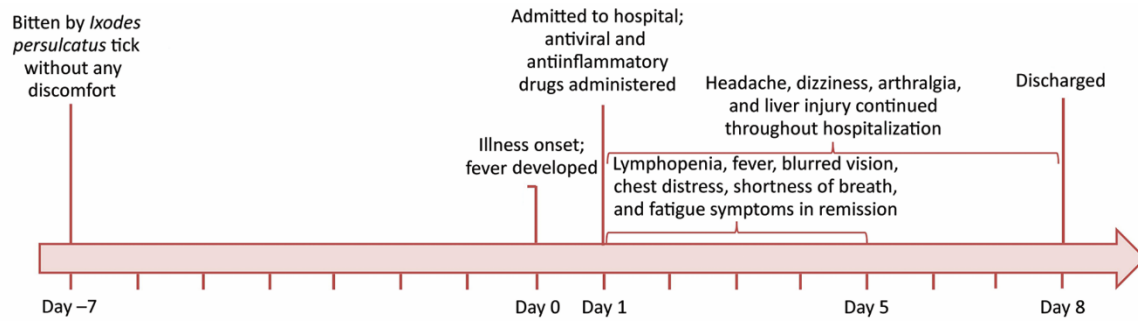
Isolate	T-HLJ01	T-HLJ02	T-HLJ03	T-IM01	T-JL01	H-IM01	HH001–20	HH003–20	HH011–20	SLNV	JANV	SGLV	TCTV1
T-HLJ01	***	99.8	99.8	99.7	99.8	99.8	99.7	99.8	99.7	82.5	45.8	44.9	44
T-HLJ02	98.4	***	99.7	99.7	99.7	100	99.8	99.7	99.7	82.4	45.7	44.8	44
T-HLJ03	98.9	98.4	***	99.6	99.8	99.7	99.6	99.7	99.6	82.4	45.8	44.8	43.9
T-IM01	98.2	98.5	98.3	***	99.6	99.7	99.7	99.7	99.6	82.3	45.8	44.8	44
T-JL01	98.8	98.3	99	98.2	***	99.7	99.6	99.7	99.6	82.4	45.7	44.8	43.9
H-IM01	98.2	98.5	98.3	100	98.3	***	99.8	99.7	99.7	82.4	45.7	44.8	44
HH001–20	97.2	97.4	97.3	97.3	97.2	97.4	***	99.7	99.8	82.5	45.7	44.8	43.9
HH003–20	98.7	98.5	98.8	98.3	98.8	98.3	97.4	***	99.6	82.4	45.7	44.8	43.9
HH011–20	97.3	97.5	97.4	97.4	97.3	97.4	98.4	97.4	***	82.5	45.7	44.8	44
SLNV	70.3	70.1	70.2	70.2	70.2	70.2	70.2	70.1	70.1	***	44.9	43.8	43.7
JANV	51	51.1	50.9	51.1	51	51.1	51.1	51	51.2	51	***	84	63.6
SGLV	50	50	50	50	50.1	50	50.1	50.1	50.1	50.5	72.9	***	63.2
TCTV1	49.5	49.6	49.5	49.6	49.5	49.6	49.6	49.5	49.6	49.4	61.1	61.6	***

*YEZV, Yezo virus; SLNV, Sulina virus strain IxriSL16–03; JANV, Ji'an nariovirus strain NE-JA; SGLV, Songling virus strain HLJ1202; TCTV1, Tacheng tick virus 1 strain TC253. YEZV strains were replaced with the strain name.

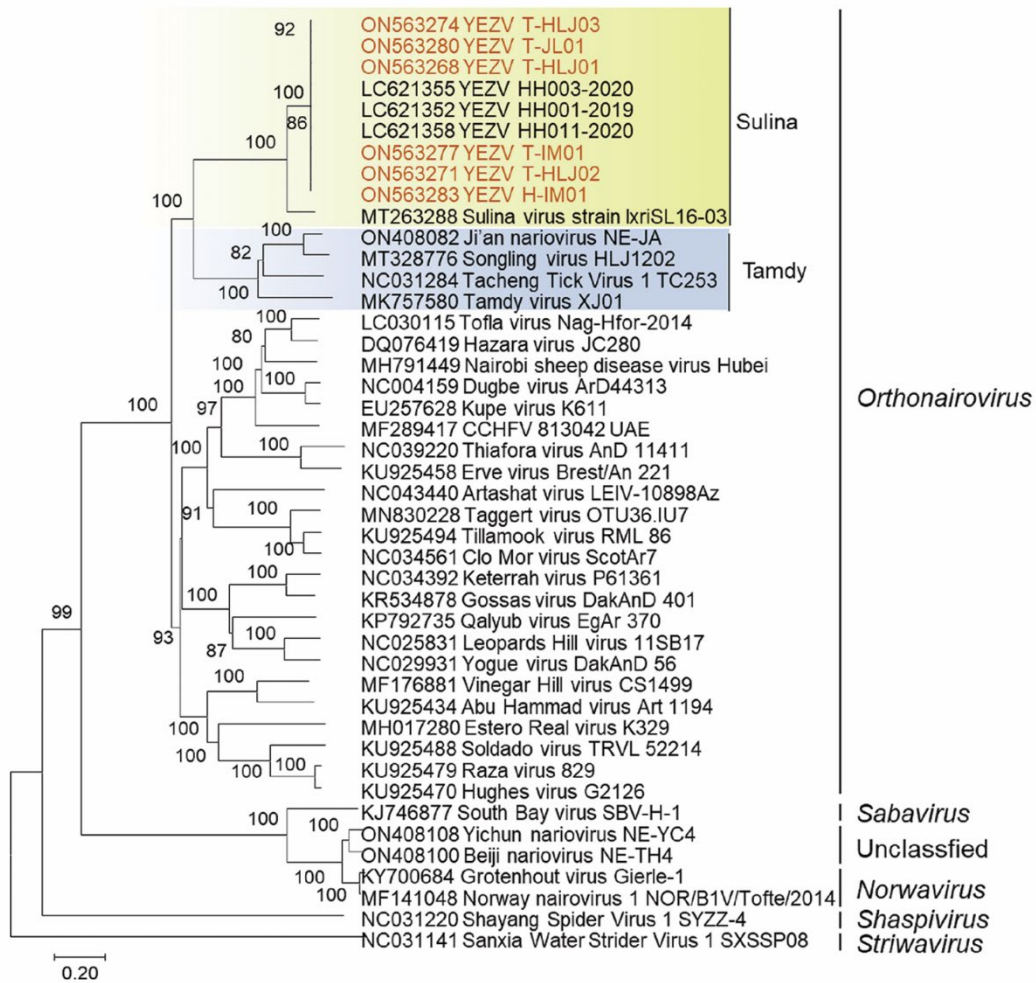
Appendix Table 5. Nucleotide sequence identities of small (lower left) and medium (upper right) segments of YEZV*

Isolate	T-HLJ01	T-HLJ02	T-HLJ03	T-IM01	T-JL01	H-IM01	HH001–20	HH003–20	HH011–20	SLNV	JANV	SGLV	TCTV1
T-HLJ01	***	98.6	98.8	98.3	98.8	98.3	97.8	98.7	97.4	59.9	43.5	43.4	43.1
T-HLJ02	98.6	***	98.4	98.6	98.4	98.6	97.9	98.5	97.6	59.9	43.5	43.7	43.2
T-HLJ03	98.8	98.4	***	98.2	98.8	98.2	97.7	98.7	97.3	59.9	43.4	43.6	43.2
T-IM01	98.3	98.6	98.2	***	98.2	100	97.8	98.2	97.5	59.7	43.4	43.4	43.3
T-JL01	98.8	98.4	98.8	98.2	***	98.2	97.7	98.6	97.4	59.8	43.4	43.5	43.2
H-IM01	98.3	98.6	98.2	100	98.2	***	97.8	98.2	97.5	59.7	43.4	43.4	43.3
HH001–20	97.8	97.9	97.7	97.8	97.7	97.8	***	97.8	98.6	59.8	43.4	43.8	43.1
HH003–20	98.7	98.5	98.7	98.2	98.6	98.2	97.8	***	97.4	59.7	43.5	43.7	43
HH011–20	97.4	97.6	97.3	97.5	97.4	97.5	98.6	97.4	***	59.8	43.3	43.7	43
SLNV	59.9	59.9	59.9	59.7	59.8	59.7	59.8	59.7	59.8	***	40.5	41	40.6
JANV	43.5	43.5	43.4	43.4	43.4	43.4	43.4	43.5	43.3	40.5	***	70.9	54.6
SGLV	43.4	43.7	43.6	43.4	43.5	43.4	43.8	43.7	43.7	41	70.9	***	55.1
TCTV1	43.1	43.2	43.2	43.3	43.2	43.3	43.1	43	43	40.6	54.6	55.1	***

*YEZV, Yezo virus; SLNV, Sulina virus strain IxriSL16–03; JANV, Ji'an nariovirus strain NE-JA; SGLV, Songling virus strain HLJ1202; TCTV1, Tacheng tick virus 1 strain TC253. YEZV strains were replaced with the strain name.



Appendix Figure 1. Schematic organization of Yezo virus (YEZV) genome. The genome is composed of large (L), medium (M), and small (S) RNA segments, and the predicted open reading frames are shown in boxes. GPC, glycoprotein precursor; N, nucleocapsid; RdRp, RNA-dependent RNA polymerase.



Appendix Figure 2. Phylogenetic analyses of Yezo virus (YEZV). A) Amino acid sequences of glycoprotein protein of family *Nairoviridae* including YEZVs. B) Amino acid sequences of nucleocapsid protein of family *Nairoviridae* including YEZVs. Amino acid sequences of representative viral strains were downloaded from NCBI public databases and aligned together by using MEGA version 7.0. Numbers along branches are bootstrap values. Bootstrapping analyses of 1,000 replicates were conducted, and values >70 were considered significant and are shown in the trees. Scale bars indicate amino acid substitutions per site.