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Case Report

The Hidden Cause of Fever Following a Tick Bite: Retrospective Identification of Yezo Virus in a 7-Year-Old Child

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Abstract

Yezo virus (YEZV, *Orthonairovirus yezoense*) is a recently identified virus belonging to the genus *Orthonairovirus*. It has been detected in ixodid ticks and blood samples from tick bite patients in Japan, China, and Russia. Human YEZV infection is associated with acute febrile illness. We retrospectively screened 147 blood samples collected from tick-bitten patients in the Kemerovo region, Russia, in 2025 using an in-house real-time reverse transcription PCR assay. YEZV RNA was detected in two samples, one of them was obtained from a 7-year-old girl following a tick bite and described in this study. *Borrelia burgdorferi* s. l. DNA was initially detected in the removed tick, and post-exposure prophylaxis with doxycycline was initiated. The patient developed a fever of unknown origin on the fifth day after the tick bite and was hospitalized on the ninth day; however, the etiology of the illness remained undetermined during the acute phase. Retrospective molecular testing subsequently identified YEZV infection as a potential cause of the disease. The main clinical manifestations included fever, malaise, asthenia, mild leukopenia, and a left shift of neutrophil count. This case provides additional evidence of YEZV circulation in Russia and highlights the need to consider YEZV infection in patients presenting with an acute febrile illness following a tick bite.

Keywords: Yezo virus; *Orthonairovirus yezoense*; tick-borne infections

1. Introduction

Yezo virus (YEZV, *Orthonairovirus yezoense*) is a recently described tick-borne virus. It belongs to the family Nairoviridae and the genus *Orthonairovirus*. YEZV was first discovered in Hokkaido, Japan [1]. Since the discovery, the known geographic distribution of YEZV has expanded considerably. Currently, YEZV has been detected in Siberia and the Russian Far East, including the Kemerovo, Khabarovsk [2], Tomsk [3], Transbaikal, Primorsky [4] regions; northeastern China, including Heilongjiang, Jilin, Inner Mongolia Provinces [5]; and Japan, including Hokkaido [6]. The primary vector of YEZV is *Ixodes persulcatus*. In addition, YEZV RNA has been detected in other tick species including *Ixodes pavlovskyi*, *Ixodes ovatus*, and *Haemaphysalis megaspinosa* [1].

YEZV causes acute febrile illness in humans. To date, 2, 22, and 23 laboratory-confirmed cases of YEZV infection have been reported among patients in Russia [2], Japan [1,6–8], and China [9], respectively. The most common clinical manifestations include fever, headache,

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malaise, dizziness, myalgia, leukopenia, thrombocytopenia, elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and decreased lymphocyte and neutrophil count.

In the present study, we retrospectively analyzed 147 blood samples collected from patients who presented to healthcare facilities after tick bites in the Kemerovo region, Russia. Two patients with YEZV infection were identified. One of these patients is described in detail in this report.

2. Materials and Methods

2.1. Study Design

A total of 147 blood samples were collected in the Kemerovo region during 2025 from 147 patients who had been bitten by a tick and presented to the Kuzbass Clinical Infectious Diseases Hospital either because of symptom onset or for preventive care. Blood samples were tested for the tick-borne pathogens most prevalent in the study region using the following commercial quantitative real-time PCR (qPCR) kits: AmpliSens *Rickettsia* spp. SFG-FL qPCR Kit; AmpliSens TBEV, *B. burgdorferi s.l.*, *A. phagocytophilum*, *E. chaffeensis/E. muris*-FL qPCR Kit; and AmpliSens *Borrelia miyamotoi*-FL qPCR Kit (AmpliSens, CRIE, Moscow, Russia). After completion of the initial testing, all 147 blood samples were retrospectively analyzed for the presence of YEZV RNA. Two samples from two different patients tested positive for YEZV RNA and were subsequently sequenced for phylogenetic analysis.

The medical history of the 7-year-old girl included a tick bite that had occurred 9 days prior to hospital admission and blood sample collection. The tick was tested for the same panel of tick-borne pathogens as described above for the blood samples. The tick was not retained for further analysis.

2.2. Yezo Virus RNA Detection

Total RNA was extracted from 1 mL serum from each of the 147 patients using the MAGNO-Sorb Kit (AmpliSens, CRIE, Moscow, Russia). All RNA samples were subsequently examined for the presence of Yezo virus RNA by an in-house two-step RT-qPCR assay. The fluorescence signal was detected using a Rotor-Gene real-time PCR cycler (Qiagen, Hilden, Germany). Details of the assay design, including primer and probe sequences and amplification conditions, have previously been reported [2].

2.3. Sequencing and Phylogenetic Analysis

The two samples positive for YEZV were subjected to genome amplification using in-house primers, following the previously described protocol [2]. cDNA libraries for high-throughput sequencing were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Paired-end sequencing was carried out on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

The raw sequencing reads underwent quality filtering and adapter removal with Trimmomatic (v0.39) using the following settings: LEADING:7, TRAILING:7, SLIDINGWINDOW:4:15, MINLEN:40, and the ILLUMINACLIP option specifying the adapter sequence file [10]. Primer trimming was then performed with cutadapt v4.9 using the non-internal adapters option [11]. Read mapping was performed using bowtie2 v2.5.4 with the local alignment option against references from GenBank PX219864.1, PX219865.1, and PX219866.1 for the L, M, and S segments, respectively [12]. SAMtools v1.23.1 was used to process and sort BAM files and generate consensus sequences [13]. The consensus sequence assembly was performed using the samtools consensus command with parameters "-aa -m simple -A -H 0.25 -d 20". Sequences identified in this study were deposited in the GenBank database (accession numbers QB057590–QB057594).

For comparative phylogenetic analysis, the newly obtained sequences were analyzed alongside previously published Yezo virus sequences retrieved from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov). Multiple sequence alignment was performed in MEGA version 12 [14]. Maximum-likelihood (ML) phylogenies were reconstructed separately for each genomic segment, using 1000 bootstrap replicates and the nucleotide substitution model with the lowest Bayesian information criterion (BIC) score. The selected models were K2 + I for the S segment (partial ORF, 823 nt), T92 + G + I for the M segment (partial ORF, 2913 nt), and GTR + I for the L segment (partial ORF, 6841 nt). The phylogenetic tree was visualized in Interactive Tree of Life (iTOL) version 7 [15].

3. Results

A 7-year-old girl was bitten by a tick while playing at a stadium in the Kemerovo region, Russia. The tick was removed from the temporal region of the scalp and tick-borne encephalitis immunoglobulin was injected on the same day the patient had been outdoors. The tick was tested for the tick-borne pathogens most common in this region, including tick-borne encephalitis virus, *Borrelia burgdorferi* sensu lato., *Anaplasma* spp., *Ehrlichia chaffeensis*, *Ehrlichia muris*, *Rickettsia* spp. *Borrelia burgdorferi* sensu lato DNA was detected in the removed tick using qPCR. On the day following the tick bite, post-exposure prophylaxis with doxycycline (100 mg once daily) was initiated. On the fifth day after the tick bite, the patient developed a febrile illness accompanied by weakness, with the body temperature rising to 38.8 °C. The timeline of the clinical course is shown in Figure 1. During outpatient evaluation, a chest radiograph was obtained, and no abnormalities were observed. Laboratory investigations revealed leukopenia (3.23×10^9/L) with a left shift of neutrophils (17% immature and 31% mature neutrophils). The patient lived in an urban apartment and did not leave the Kemerovo region during the 3 months preceding the tick bite. Her medical history included urinary tract infection, viral respiratory infections, giardiasis, chickenpox, bronchitis, and rotavirus infection.

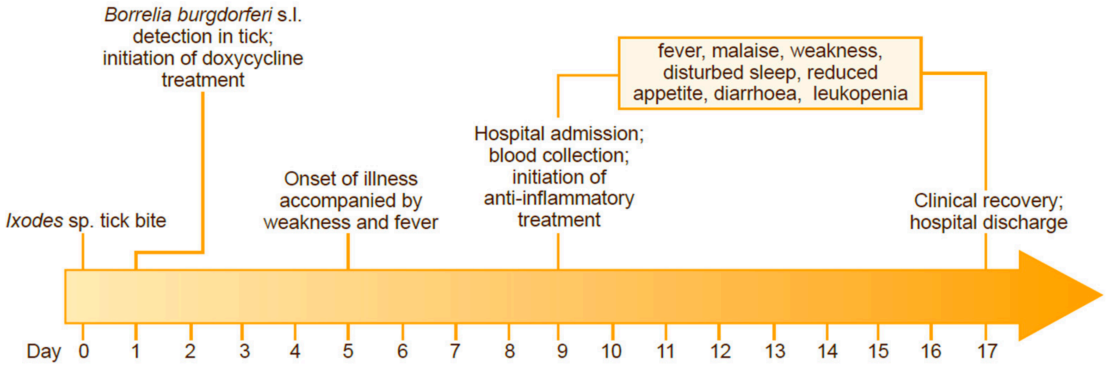


Figure 1. Timeline of the clinical course of the child with Yezo virus infection in the Kemerovo region.

On the ninth day after the tick bite, the patient was admitted to hospital with malaise. Her general condition was of moderate severity, and her body temperature was 38 °C. Sleep was disturbed, and appetite was reduced. No skin rash was observed. The pharyngeal mucosa was erythematous with no coating. The anterior cervical and submandibular lymph nodes were enlarged and non-tender. Nasal breathing was obstructed; no wheezing in the lungs was observed. No meningeal symptoms were present. The patient had multiple episodes of diarrhea, while urination remained normal. The Clinical Dehydration Scale (CDS) score was 4, consistent with mild dehydration.

Chest radiography, electrocardiography, and ultrasonography of the kidneys and adrenal glands revealed no abnormalities. Blood tests on day 9 after the tick bite revealed mild leukopenia with a white blood cell count of 3.29 × 109/L, elevated lymphocyte ratio of 40%, elevated immature neutrophil ratio of 10%, and reduced mature neutrophil ratio of 40%. Complete blood test results are presented in Table S1. The patient was discharged in satisfactory condition on day 17 after the tick bite.

During hospitalization, the etiology of the febrile illness remained undetermined. On the day of admission, a blood sample was collected and tested by quantitative real-time PCR (qPCR) for the presence of the tick-borne pathogens most prevalent in the study region, including *Rickettsia* spp., *TBEV, B. burgdorferi* s.l., *A. phagocytophilum*, *E. chaffeensis/E. muris*, and *Borrelia miyamotoi*. All qPCR assays yielded negative results. Following the detection of *Borrelia burgdorferi* s. l DNA in the removed tick, doxycycline therapy was continued. Empirical antibacterial therapy with ceftriaxone was administered along with intravenous fluid therapy to correct the dehydration. Rectal interferon alfa-2b and normal human immunoglobulin were also administered. Further details of the treatment are provided in Table S2.

The blood sample collected from this patient on day 9 after the tick bite was included in a retrospective analysis of 147 blood samples collected from patients who presented to the hospital after a tick bite in the Kemerovo region in 2025. An in-house real-time RT-PCR (RT-qPCR) assay previously described [2] was used to detect Yezo virus RNA. YEZV RNA was identified in two samples. The first blood sample was obtained from a patient for whom no detailed clinical information was available because the patient was not hospitalized and received treatment at home. The RT-qPCR Ct value was 24.4. The second blood sample was collected from the 7-year-old girl whose clinical case is described above. The RT-qPCR Ct value was 29.8. Repeat RT-qPCR analysis was not performed on either of the YEZV-positive samples. These findings identified a case of Yezo virus infection in a child following a tick bite.

The YEZV-positive blood samples obtained from a 7-year-old girl and from another patient not described in this study were included in the subsequent phylogenetic analysis (Figure 2). High-throughput sequencing of partial open reading frames (ORFs) of all three YEZV genome segments was conducted. For the 7-year-old girl, the following sequence lengths were obtained: 1436 nt for the S segment, 2872 nt for the M segment, and 6824 nt for the L segment. For the other patient, sequences of 812 nt and 3872 nt were obtained for the S and M segments, respectively. Sequences obtained in this study have been deposited in GenBank (accession numbers QB057590–QB057592 for the child described in this study and QB057593–QB057594 for the other patient). Sulina virus was selected as the outgroup. Across all three genomic segments, the obtained sequences clustered within the same clade as the sequences from Kemerovo reported in our previous study [2].

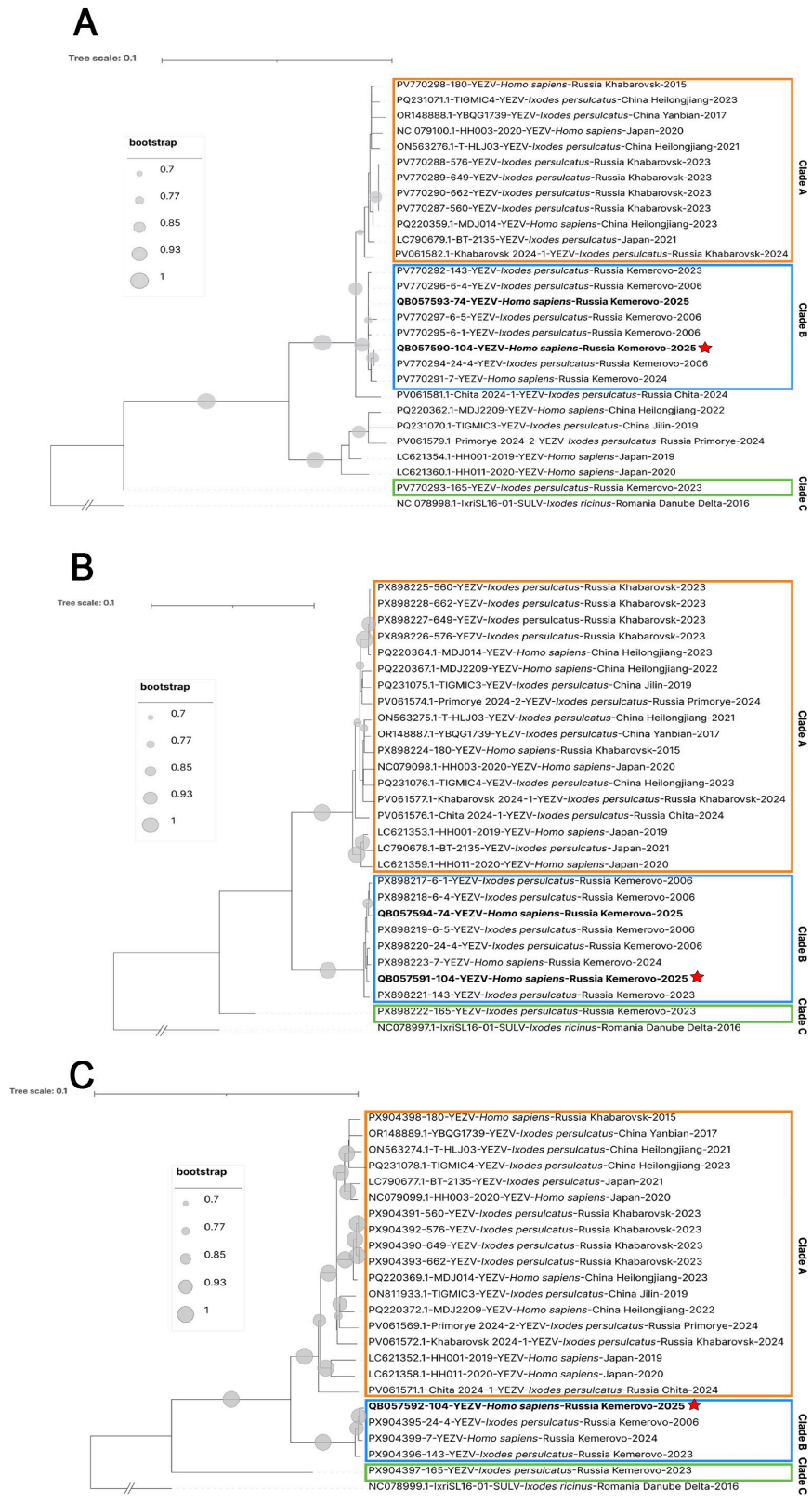


Figure 2. Phylogenetic relationships among the Yezo virus S (**A**), M (**B**), and L (**C**) genomic segments. The analyzed ORF fragments comprised 823, 2913, and 6841 nt, respectively. Sequences obtained in this study are shown in bold; a star identifies the isolate from the 7-year-old patient. Reference sequences were retrieved from GenBank and analyzed in MEGA 12 using maximum-likelihood inference with 1000 bootstrap replicates. The substitution models were K2 + I (S), T92 + G + I (M), and GTR + I (L). Bootstrap support values exceeding 0.7 (expressed as proportions) are marked with gray circles.

4. Discussion

This case represents the youngest patient reported with Yezo virus (YEZV) infection. To date, no cases of YEZV infection in children have previously been reported. The reported median age of patients with YEZV infection was 59.5 years in Japan [6] and 53 years in China [9]. In our previous study, the two patients with YEZV infection were 68 and 69 years old [2]. The present case suggests that children may also be susceptible to YEZV infection.

Preventive antibiotic treatment was initiated immediately after *Borrelia burgdorferi* s. l. DNA was detected in the removed tick. Therefore, the negative result of the patient's blood test for *Borrelia burgdorferi* s. l. DNA was considered inconclusive. In our case, *Borrelia burgdorferi* s. l. DNA was detected in the tick, whereas YEZV RNA was detected in the patient's blood. Thus, we could only hypothesize that the patient had a coinfection; however, this could not be confirmed. Coinfections involving Yezo virus and other tick-borne pathogens have been described in previous reports. A case of YEZV coinfection with *Borrelia miyamotoi* has been reported in Japan [8]. Similarly, our previous study documented a case of YEZV coinfection with *Rickettsia heilongjiangensis* in Russia [2]. In the present case, doxycycline treatment was initiated immediately after the tick bite. YEZV infection may have contributed to the clinical manifestations observed in the patient. These manifestations included fever, malaise, weakness, disturbed sleep, reduced appetite, diarrhea, and leukopenia with a left shift of neutrophils. Similar clinical and laboratory findings have been reported in patients with YEZV infection in previous studies [2,6,9].

However, although the symptoms described above are consistent with YEZV infection, their presence does not necessarily indicate an infection with YEZV, as similar clinical manifestations may occur in other tick-borne diseases. In particular, infections caused by recently identified orthonairoviruses, including Songling virus (SGLV) and Wetland virus (WELV), may present with a similar nonspecific febrile illness [16,17]. SGLV infection has been associated with headache, fever, depression, fatigue, dizziness, myalgia, arthralgia, rash, anorexia, and nausea [16]. A similar clinical presentation has been reported in patients with WELV infection, including fever, dizziness, headache, malaise, myalgia, arthritis, and back pain. Laboratory abnormalities observed in WELV infection include leukopenia, lymphopenia, and thrombocytopenia [17]. The potential geographic overlap of these infections further underscores the importance of differential diagnosis. SGLV RNA has been detected in several ixodid tick species in China, including *Ixodes crenulatus*, *Ixodes persulcatus*, *Haemaphysalis concinna*, and *Haemaphysalis longicornis* [16]. Recent surveillance has also demonstrated SGLV RNA in *H. concinna* ticks in Russia [18]. WELV RNA has been detected in *H. concinna*, *Haemaphysalis japonica*, *I. persulcatus*, *Dermacentor silvarum*, and *H. longicornis* [17]. The clinical overlap is not limited to orthonairovirus infections. Lyme borreliosis, caused by *Borrelia burgdorferi* s. l may also present with nonspecific symptoms, including fatigue, fever, malaise, headache, myalgia, arthralgia, and rash [19].

In our recent study, we demonstrated a high genetic diversity of YEZV in Russia compared with strains reported in studies from Japan and China [2]. Crimean-Congo hemorrhagic fever virus (CCHFV), which like YEZV belongs to the genus *Orthonairovirus*, also shows a high level of genetic diversity. A previous study reported an association between CCHFV genetic lineage and disease severity. In particular, infection with a CCHFV genetic variant belonging to the Europe-1 lineage was associated with an increased risk of severe clinical manifestations, hemorrhagic syndrome, and fatal outcomes compared with infections caused by a CCHFV genetic variant belonging to the Europe-3 and Africa-3 lineages. However, the authors noted that the small number of cases associated with the Europe-3 and Africa-3 lineages and with some genetic variants within Europe-1 limited these comparisons [20]. Whether particular YEZV genetic variants are associated with differences

in clinical manifestations remains unknown, largely because of the limited number of reported cases, particularly those involving the genetic variants from the Kemerovo region.

It remains challenging to determine the causative tick-borne pathogen based on clinical manifestations alone, as these manifestations often overlap among different tick-borne infections. Identification of the tick species may help narrow the range of potential pathogens transmitted by the tick. Real-time PCR followed by sequencing provides a useful approach to pathogen identification. Even when a tick transmitted pathogen is identified, testing for other relevant pathogens may be appropriate because of the possibility of coinfection. Therefore, both the tick and the patient's blood sample may be tested using a pathogen panel that includes YEZV. It remains unclear whether YEZV infection is associated with any long-term effects as it is a relatively recently recognized infection, and fewer than 50 laboratory-confirmed cases have been reported to date. Long-term monitoring of patients after recovery may therefore be considered.

5. Conclusions

This report describes the first documented case of YEZV RNA detection in a child, expanding the known age range of individuals in whom YEZV has been identified. YEZV should be considered in the differential diagnosis of febrile illnesses of unknown etiology, particularly given the possibility of coinfection with other tick-borne pathogens.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens15111210/s1. Table S1: Laboratory blood test results of the 7-year-old girl hospitalized after a tick bite in the Kemerovo region. Table S2: Treatment prescribed for the 7-year-old girl before and during hospitalization after a tick bite in the Kemerovo region.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study and from the patient's parent. Informed consent for publication was obtained from the patient's parent.

Data Availability Statement: The data presented in this study are available from the NCBI (National Center for Biotechnology Information) GenBank at https://www.ncbi.nlm.nih.gov/nucleotide (accession numbers QB057590-QB057594).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

YEZV	Yezo virus
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ORF	Open reading frame
SGLV	Songling virus
WELV	Wetland virus
CCHFV	Crimean-Congo hemorrhagic fever virus

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