Epidemiological, clinical, and virological characterizations of Yezo virus infections, an emerging tick-borne orthonairovirus disease in Japan

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Contributions

HY and KMat contributed to the conceptualization, data curation, and funding acquisition. HY, KMiz, KW, YOh, KMit, and KT contributed to the analysis, investigation, visualization, and methodology. NK and YOr contributed to the resources. HY and KMiz contributed to the writing – original draft. MS and KMat contributed to the supervision and writing – review & editing. All authors had full access to and verified the study data, contributed to the final manuscript, and were involved in the decision to submit the manuscript.

Abstract

Background

Febrile disease caused by infections of Yezo virus (YEZV), a tick-borne orthonairovirus, was first reported in Japan in 2021 and subsequently identified in China. While the recent studies suggest a broader endemic area of the emerging YEZV infections, its clinical and epidemiological characteristics remain poorly elucidated due to the limited number of reported cases.

Methods

We conducted retrospective and prospective surveillance of patients with suspected tick-borne febrile illnesses in Hokkaido, Japan, between 2013 and 2024. Serum samples were analyzed by RT-qPCR, IgM/IgG ELISA, and neutralization assay. Viral genome of isolates was sequenced and phylogenetically analyzed with publicly available YEZV genomes.

Results

A total of 22 patients with YEZV infections including 10 newly identified patients was analyzed. Most infections occurred between May and July, coinciding with the peak activity of *Ixodes* ticks in the study area. A case from southern Hokkaido was firstly identified. Fever, leukocytopenia, thrombocytopenia, elevated levels of liver enzymes, and ferritin were determined as common features observed in patients with YEZV infections. Viral RNA was typically detectable in the serum within 7 days after the disease onset. Serum antibodies became positive later and might persist up to at least 600 days. Phylogenetic analysis incorporating five new isolates showed the circulations of two genetic groups of YEZVs in Japan and China, respectively.

Conclusions

Our results revealed characteristics of YEZV infections in patients and highlighted the importance of combination of molecular and serological diagnostics. The proposed case definitions and diagnostic framework may contribute future surveillance.

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Background

Emerging and re-emerging tick-borne viral diseases have been becoming a significant public health concern due to continuous identifications of several tick-borne viruses causing febrile and/or neurologic illness [1] . Nairoviruses are negative-stranded RNA viruses generally transmitted by tick vectors and include Crimean–Congo hemorrhagic fever virus (CCHFV), which causes CCHF, one of the well-known viral hemorrhagic fever with high case fatality rate. Since 2020, seven emerging and re-emerging tick-borne nairoviruses have been identified in East Asia: Tamdy virus (*Orthonairovirus tomdiense*) [2,3] , Tacheng tick virus-1 (*Orthonairovirus tachengense*) [4,5] , Beiji nairovirus (*Norwavirus beijiense*) [6] , Songling virus (*Orthonairovirus songlingense*) [7] , Wetland virus (unclassified) [8] , Xue-Cheng virus (unclassified) [9] , and Yezo virus (YEZV; *Orthonairovirus yezoense*) [10] . YEZV infections in humans were first reported in Japan in 2021, where seven patients with a febrile disease with leukocytopenia and thrombocytopenia were identified in 2014-2020 [10] . Subsequently, a retrospective YEZV infection case in China was recognized [11] . To date, a total of 12 and 23 YEZV infection cases have been reported in northern Japan [12–16] and northeastern China [17,18], respectively.

Geographical distribution of YEZV in ticks has been confirmed by genetic detections and/or infectious virus isolation mainly from *Ixodes persulcatus* ticks in Japan [19] , China [11,17,18,20,21] , and Eastern Siberia, Russia [22] . Moreover, YEZV genomes have been detected in *I. persulcatus* ticks on migratory birds captured at Hokkaido, Japan, which travel across Eastern Asian countries [23] . Since the wide distributions of *I. persulcatus* across the northern countries of the Eurasian continent [24] ,

YEZV may be more widely disseminated than recognized, suggesting undiagnosed human patients with YEZV infections in the outside of the current endemic areas. Thus, characterization of the clinical manifestations associated with YEZV infections and establishment of diagnostic criteria are necessary for accurate diagnosis as well as better understanding of the epidemiology of YEZV infections in order to clarify the geographic range of YEZV distributions.

Here, we conducted a retrospective and prospective surveillance using serum samples collected from patients suspected of having tick-borne diseases in Hokkaido during 2013–2024. This study is a follow-up to our previous investigations, in which two case and five retrospective patients in 2014–2020 were reported [10] , and also four case reports [13–16], where the dates of disease onset are available. In the present study, we identified 10 additional YEZV-infected patients in Hokkaido and genetically analyzed five YEZV isolates, including one isolate from a tick attaching to one of the patients. We comprehensively summarized the epidemiological characteristics, patient demographics, and clinical features including symptoms and laboratory findings, for a total of 22 patients. Furthermore, to better understand the diagnostic performance of available methods, we performed genetic, serological, and neutralization tests on YEZV-positive sera, including some paired samples. The genetic analysis using all the currently available YEZV genome sequences including five additional present isolates, clarified the up-to-date genetic relationships among current YEZVs. This study integrates clinical and phylogenetic data to improve understanding of YEZV infection and to support the development of future diagnostic and surveillance strategies for YEZV infection cases.

Implications of all the available evidence

Our study provides the largest clinical series of YEZV-infected patients to date, offering critical insights into its clinical presentation, laboratory characteristics, and optimal diagnostic strategies. The identification of sustained viral circulation since at least 2014, along with evidence of genetic similarity among strains from Japan, China, and Russia, highlights the importance of international surveillance efforts. The observed overlap in symptoms and laboratory findings with other tick-borne febrile illnesses emphasizes the necessity of combined molecular and serological diagnostics. The proposed case definitions and testing strategy can serve as a practical foundation for future epidemiological investigations and public health responses to YEZV infections.

Methods

Study design and biological samples

A total of 388 serum samples were collected between 2013 and 2022 from patients suspected of having tick-borne diseases for testing the notifiable diseases (including tick-borne encephalitis [TBE], severe fever with thrombocytopenia syndrome [SFTS], Lyme disease, and *Borrelia miyamotoi* disease [BMD]) and used for the retrospective surveillance (Fig. 1). These samples included those from previously reported cases of YEZV infections [10,13–16]. For prospective surveillance, a total of 163 serum samples were newly obtained in 2023 and 2024, which were collected from patients suspected of having tick-borne diseases including YEZV infection. All samples used in both retrospective and prospective surveillance were obtained from hospitals in Hokkaido with anonymous information of the patient including suspected date and place

of tick bite, date of disease onset, symptoms, and laboratory findings. For testing YEZV infections, YEZV RNA and specific antibodies were detected by quantitative RT-PCR (RT-qPCR) and IgM/IgG ELISA, respectively. Data regarding clinical manifestations, underlying medical disorders, laboratory test results, treatment, and outcomes were retrieved from physicians' reports. In addition to the above 551 serum samples, paired sera were obtained from 9 YEZV-positive patients whose clinical courses could be followed. All participants enrolled in the prospective surveillance gave written informed consent, and ethical approval was obtained from the ethics committee of Hokkaido Institute of Public Health.

YEZV genome detection

Total RNA was extracted from patient serum samples using QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturers' protocols. Detections of YEZV S segment RNA were performed using One Step PrimeScript III RT-qPCR mix (Takara) and PrimeTime qPCR assays (primer 1: 5'-AGCCCTTGACACTGCATTT-3', primer 2: 5'-CATACAGGAAGGCCATCTCATT-3' and probe: /56-FAM/ACCTACTAC/ZEN/TGGATGTGGAAGGCAGA/3IABkFQ/) (Integrated DNA Technologies) on a StepOnePlus Real-Time PCR system (Thermo Fisher Scientific) with the following conditions; 52 °C for 5 min, 95 °C for 10 s, and 40 cycles of 95 °C for 5 s and 60 °C for 30 s. A standard curve was generated using a series of diluted plasmids carrying a fragment of YEZV S segment cDNA.

YEZV-specific antibody detection

The detections of IgM and IgG antibodies recognizing YEZV were performed by ELISA as previously reported [10] . In brief, heat-inactivated serum was serially diluted and applied to the YEZV nucleoprotein (N) antigen-coated 96-well ELISA plate, followed by the reaction with the secondary antibodies; goat anti-human IgM mu chain (HRP) preabsorbed or rabbit anti-human IgG H&L (HRP) (Abcam). 3,3',5,5'-tetramethylbenzidine (TMB) liquid substrate system for ELISA peroxidase substrate (Merck) was used to visualize the YEZV N specific antibodies, and samples were defined as positive when the ratio of absorbances between YEZV N antigen and mock antigen was >1.3. The reciprocal of the highest serum dilution in which the serum sample was positive was used as the serum antibody titer.

Serum neutralization antibody detection

Serum neutralization antibody titers against YEZV were determined by focus reduction neutralization test (FRNT). Serum samples were 10-time diluted with Dulbecco's Modified Eagle Medium (DMEM; Nacalai Tesque, Japan) containing 2% fetal bovine serum (Sigma-Aldrich) and 100 μg/ml penicillin and streptomycin (Wako, Japan) (2% FBS DMEM) followed by 2-fold serial dilution with 2% FBS DMEM. 100 focus forming units (FFU) of YEZV strain HH003-2020 [10] was incubated with the equivalent volume of a serum dilution at room temperature for 1 h. Vero E6 cells seeded at 1×10^5 cells/well in a 24-well plate a day before the inoculation, were inoculated with YEZV–serum mixtures at 37°C with 5% CO_2 for 1 h. Then, the virus inoculum was removed and washed with D-PBS(-) (Nacalai Tesque) twice and replaced with Minimum Essential Medium containing 2% FBS, 100 μg/ml of penicillin and streptomycin, and 1% methylcellulose

(Sigma-Aldrich). Cells were incubated at 37°C with 5% CO_2 for 3 days, and fixed with 4% paraformaldehyde at room temperature for 30 min. Fixed cells were washed with D-PBS(-) twice and incubated with the YEZV-infected mouse serum diluted 1:1,000 in blocking and permeabilization buffer (0.3% Triton X-100 [Sigma-Aldrich] in PBS containing 3% bovine serum albumin [Wako]) at room temperature for 1 h. The cells were then washed with D-PBS(-) for three times. Virus antigen-antibody complexes were visualized using rabbit anti-human IgG (H + L) conjugated with horseradish peroxidase (Abcam) and ImmPACT VIP (Vector Laboratories). Visible foci were counted manually, and serum samples presenting 50% focus reduction than the negative control human serum were considered as FRNT positive.

Virus isolation and sequencing

Type-I and -II interferon-receptor double knockout mice (AG129 mice, Marshall BioResources, Japan) were inoculated with the YEZV RNA-positive patient sera diluted at 1:10 by normal saline (Otsuka Pharmaceutical Factory, Japan) and inoculated into at 100 µl/head, intraperitoneally. At two days after inoculation, the blood was collected by cardiac puncture for obtaining the mouse serum. Another AG129 mice were inoculated with the serum for passaging when necessary. Ticks removed from YEZV-positive patients were homogenized in PBS without KCl (pH 7.2) (Nacalai tesque) and used for virus isolation by inoculating AG129 with the homogenate as above. Using RNA samples extracted from the sera collected from the AG129 mice, sequencing libraries were prepared using the KAPA RNA hyper prep kit (for Illumina) and the KAPA dual-indexed adapter kit (Roche). The prepared libraries were sequenced on an MiSeq and NextSeq

1000 sequencer using the MiSeq reagent kit v3 and NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (600 Cycles) (Illumina) with 2 × 300-bp paired-end read length, respectively. Sequencing reads were *de novo* assembled using the CLC genomics workbench ver. 23 (Qiagen). Identified genome sequences were deposited on GenBank (Appendix Table S1).

Phylogenetic analysis

For phylogenetic analysis, YEZV whole genome sequences were obtained from GenBank. The nucleotide sequences in coding regions of tripartite YEZV RNA segments (N, glycoprotein precursor (GPC), and RNA-dependent RNA polymerase (RdRp)) were aligned according to the translation align with outgroup sequences (N: Sulina virus, NC_078998, GPC and RdRp: YEZV THQG1707B) by MAFFT v7.490 in Geneious Prime, respectively, and the start and stop codons were manually excluded to prevent the selection of an inappropriate nucleotide substitution model. The phylogenetic trees by maximum likelihood inference for three genes were constructed using IQ-TREE 2.4.0 [25] in each gene tree. The nucleotide sequences were partitioned according to codon position, and a substitution model for each position was selected by ModelFinder [26] using the -m MFP option implemented in IQ-TREE. The nodal support values were calculated using 10000 replicates with the SH-like approximate likelihood ratio test (SH-aLRT) [27] and ultrafast bootstrap (UFBoot) [28] methods. The constructed trees were visualized by TreeViewer v2.2.0 [29] .

Ethics statement

For the retrospective surveillance, patient information was handled using an opt-out method, in which study details were disclosed and patients had the opportunity to decline participation. For the prospective surveillance, serum samples were collected and used for virologic analysis after obtaining written informed consent from the patients themselves. All protocols and procedures were approved by the research and ethics committees of the Hokkaido Institute of Public Health (approval numbers: E19-05, E20-05, and E23-09) and Hokkaido University (approval number: 023-0159), respectively.

Results

To investigate the latest epidemiological situation of YEZV infections in Hokkaido (Fig. 2A), we conducted both retrospective and prospective surveillance. A total of 388 (retrospective) and 163 (prospective) samples were used for the viral RNA and antibody detection (Fig. 1). We identified 19 seropositive samples (3.4%) and 18 RNA-positive samples (3.3%) (Table 1). Of these, 15 samples (2.7%) were positive for both viral RNA and antibodies, and thus, 22 individuals who tested positive for YEZV RNA and/or antibodies were identified as YEZV-infected cases. The number of YEZV cases were shown by reported regions and year (Fig. 2B), and month (Fig. 2C) of disease onset for available 20 YEZV-positive cases. A case of YEZV infection was identified for the first time in southern Hokkaido in 2024. The disease onset of all the cases were from May to July.

Two cases with only IgG positive during the acute phase (#19 and 22 in Supplemental Table S2) have been removed from the following analyses since the disease onsets of YEZV infections could not be verified. Among the 20 confirmed YEZV

infection cases, 15 (75.0%) were male and 5 (25.0%) were female (Table 2). The median age was 59.5 years (interquartile range [IQR]: 40.3-71.5 years) ranging from 22 to 80 years of age. Underlying conditions included hypertension and dyslipidemia, each observed in three patients (15.0%). Hyperuricemia, diabetes mellitus, polymyalgia rheumatica, behavioral disorder, and Hirschsprung's disease were also observed in one patient each. A total of nine patients (45.0%) was administered antibiotics due to suspected infections with *Borrelia* spp. Six out of nine patients were reported to test positive for *Borrelia* spp. antibodies. A total of 18 patients (90.0%) had a history of tick bite within two weeks prior to fever onset. The median estimated incubation period from tick bite to fever onset was 7 days (IQR: 6–9) (Fig. 2D).

Among patients whose clinical data were available, 19 patients (95.0%) presented with fever, and 10 out of 14 patients with actual body temperature reports (71.4%) presented with high fever of >38.0°C (Table 3). The most common symptom was headache (60.0%). Fatigue and myalgia/arthralgia were also common symptoms with onset rates of 50.0%. A total of 10 patients (50.0%) presented with at least one gastrointestinal manifestation, including nausea, abdominal pain, diarrhea, and anorexia. One patient (5.0%) presented with lymphadenopathy. Notable respiratory and hemorrhagic symptoms were not reported. All patients (100%) presented with thrombocytopenia. Leukocytopenia (95.0%) and elevation of hepatic enzymes (85.0%) were also commonly presented. The elevation levels of aspartate aminotransferase (100%), alanine aminotransferase (85.7%), and ferritin (100%) were often found. In addition, a part of patients presented with the elevation levels of lactate dehydrogenase (72.7%), creatine kinase (33.3%), and C-reactive protein (18.2%).

To assess the temporal viral testing window of YEZV infection, we presented the YEZV RNA and antibody detection outcomes of each diagnostic method used in our surveillance according to days post fever onset (Fig. 3, appendix Table S2). Serum samples were collected up to 40 days after fever onset from 17 YEZV-positive patients, whose onset dates (Day 0) were recorded. Twenty-five samples were collected from their acute phase of disease course, and 28 convalescent-phase sera were obtained from 10 patients who visited the same hospitals for the follow-up examination. YEZV RNA was detectable from Day 2 to Day 19 (median: 6.5 days), with 94.1% (16/17) of patients testing RT-qPCR positive with their initial samples. IgM antibodies, as measured by ELISA, were detectable from 5 days after the onset, while only one sample at Day 5 (25.0%; 1/4) was positive. IgG antibodies were detected in samples collected after Day 8, although negative samples included those collected before Day 16. To demonstrate the YEZV specific antibodies in serum, neutralizing antibodies were also measured by FRNT (Fig. 3A). The detection window of neutralization antibodies was almost identical to that of the IgG ELISA. For further analyzing the serum neutralizing antibody responses in YEZV-infected patients, we obtained available serum samples during the recovery phase of patients. With eight patients those who tested positive for RT-qPCR, seroconversion was identified by FRNT (Fig. 3B). With a patient whose serum samples could be obtained after the recovery until Day 613, FRNT was performed to examine the duration of antibody seropositivity (Fig. 3C). Neutralizing antibodies were detected from Day 10 through Day 613, with fluctuations in titer levels, suggesting that YEZV infection may induce a humoral immune response that persists for over two years.

We finally performed virus isolation from patient sera and ticks infesting to

patients. Four isolates were obtained from sera and one from a tick. Unfortunately, we could not obtain YEZV isolates from a combination of a patient and a tick biting to the patient. The tripartite RNA segments of the isolates were sequenced, and phylogenetic analysis was conducted alongside 38 reference YEZV sequences retrieved from the public database (Fig. 4). We defined grouping of the phylogenetic clusters following the previous research based on the L-segment tree [17]; clade I-C, I-J, II-C, and II-J. The clade I-C and II-J, corresponding to the previous clade I and II, respectively, included almost all isolates from the Eurasian continent, i.e. China and Russia. The present Japanese YEZV isolates from the patients and the patient-associated tick were classified into the clade I-J and II-J together with those reported in the previous studies in Japan [10,23] . No genetic reassortment has been identified in the present isolates.

Discussion

We have conducted a surveillance study of patients with suspected tick-borne febrile illnesses in Hokkaido, Japan, between 2013 and 2024, and newly identified 10 cases of YEZV infections since our previous study [10] . The total number of patients of YEZV infections in Hokkaido, Japan is 22 as of May 2025, representing the largest clinical series of YEZV-infected patients reported to date. The epidemiological characteristics of patients in Japan are similar to those reported in China with slightly older median of age, and our surveillance found the youngest case of 21 years of age. The present summary of clinical features, patient backgrounds, and diagnostic sensitivities using the Japanese patients' information are expected to contribute to future establishment of clinical case definitions and diagnostic strategies for YEZV infection surveillance.

In this study, YEZV infection was characterized as an acute febrile illness developing after an approximately a week incubation period following tick bites, presenting commonly with headache, fatigue, gastrointestinal symptoms, leukocytopenia and thrombocytopenia in total blood cell counts, and elevated hepatic transaminases, which are largely consistent with the previous reports [17] . A notable difference in the clinical characteristics between our study and the previous study reported from China was that there were no reports of patients with hemorrhagic symptoms (e.g. skin lesions such as maculopapular erythema or hemorrhagic symptoms such as petechiae) in our study. Furthermore, gastrointestinal hemorrhagic symptoms, such as bloody diarrhea and gingival bleeding, which are commonly observed in SFTS cases [30] , were not reported in the present study. Interestingly, all patients tested in the present study demonstrated elevated ferritin levels, which has also been reported in tick-borne disease cases complicated by hemophagocytic lymphohistiocytosis [31] and SFTS [32] . Taken together, the present study confirmed that YEZV infection presented with non-specific febrile symptoms, and laboratory findings of YEZV patients were similar to those observed in other tick-borne acute viral febrile diseases, especially SFTS, which underscore the importance of combining virological test for reliable diagnosis.

Because of the non-specific febrile symptoms and laboratory scores of YEZV infections, differential diagnosis with other tick-borne diseases endemic in the same region should be essential. For developing a definitive diagnostic testing strategy, we evaluated the duration in which each virological test was positive. Most samples collected within a week after fever onset tested positive for YEZV RNA by RT-qPCR. While YEZV RNA was detected up to 18 days after symptom onset in some cases, RT-qPCR shall not

be recommended to use after 10 days post disease onset, in consistent with our previous observations in two patients [10] . Thus, serological assays to detect IgM antibody responses at the acute phase and also to confirm seroconversion, should be combined with viral RNA detection at the acute phase to ensure reliable molecular testing of YEZV infections. Since neutralization antibody may persist in YEZV patients more than a year, serological assays will also be useful to retrospectively confirm past YEZV infections.

YEZV is currently identified from patients and ticks (both host-questing and attaching to humans and birds) in the Far Eastern Asia. The present study reported five new isolates and their complete genome sequences, and then constructed the latest phylogenetic trees of YEZV three segment RNAs. Since no previous studies addressed the evolutional relationships among YEZV isolates using complete sets of viral genome sequences, we applied the most accurate models to build the phylogenetic trees based on the latest published YEZV sequences including Japanese, Chinese, and Russian isolates. All the isolates identified in the present study were grouped together with the previously identified Japanese isolates. Our analysis revealed that the genetic diversity among S segment RNAs of the clade II-C isolates, which might be detected as a reassortment of the isolate T-HL02 by the previous study [23]. Furthermore, we could identify the multiple overseas transmissions of YEZVs between Hokkaido, Japan and Northern China or Russia, which could be observed as the emergences of the Japanese clades and also as a reassortant isolate YBQG1712 reported from China with M and L segments RNAs classified into the Japanese clade II-J. In addition to the possible overseas transmissions, multiple genetic reassortment events between the clades I-C and II-C or the clades I-J and II-J, highlight the active circulations of YEZVs in nature.

The present study revealed that YEZV infection is an endemic tick-borne disease throughout Hokkaido, Japan and emphasized the need for further epidemiological investigations as well as environmental research for understanding the intra- and inter-regional circulations of YEZVs. Since *Ixodes* ticks, especially *I. persulcatus* ticks, are likely to be a primary vector of the disease, YEZV infection can be more widespread across northern Eurasia than observed. By providing clinical and laboratory diagnostic evidence, our study contributes to further identifications of YEZV infections, for which differentiating diagnosis to the other endemic tick-borne diseases such as SFTS and Lyme disease and the other emerging and reemerging tick-borne orthonairovirus diseases.

Data availability

YEZV genome sequences newly identified in the present study are available at GenBank with accession numbers PX120886-PX120900. Clinical data is not publicly available.

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Conflicts of interest

All authors declared no potential conflicts of interest in relationship to this study.

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References

1. Zhou H, Xu L, Shi W. The human-infection potential of emerging tick-borne viruses is a global public health concern. Nat Rev Microbiol. **2023**; 21(4):215-217.

2. Moming A, Shen S, Fang Y, et al. Evidence of Human Exposure to Tamdy Virus, Northwest China. Emerg Infect Dis. **2021**; 27(12):3166-3170.

3. Chen S, Saqib M, Khan HS, et al. Risk of infection with arboviruses in a healthy population in Pakistan based on seroprevalence. Virol Sin. **2024**; 39(3):369-377.

4. Liu X, Zhang X, Wang Z, et al. A Tentative Tamdy Orthonairovirus Related to Febrile Illness in Northwestern China. Clin Infect Dis [Internet]. **2020**; 70(10):2155-2160. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31260510

5. Zhang Y, Jiang L, Yang Y, Xie S, Yuan W, Wang Y. A tick bite patient with fever and meningitis co-infected with Rickettsia raoultii and Tacheng tick virus 1: a case report. Bmc Infect Dis [Internet]. **2021**; 21(1):1187. Available from: https://doi.org/10.1186/s12879-021-06877-z

6. Wang Y-C, Wei Z, Lv X, et al. A new nairo-like virus associated with human febrile illness in China. Emerg Microbes Infect [Internet]. **2021**; 10(1):1200-1208. Available from: https://www.ncbi.nlm.nih.gov/pubmed/34044749

7. Ma J, Lv X-L, Zhang X, et al. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. **2021**; 27(3):434–439.

8. Zhang X-A, Ma Y-D, Zhang Y-F, et al. A New Orthonairovirus Associated with Human Febrile Illness. N Engl J Med [Internet]. **2024**; 391(9):821–831. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa2313722

9. Zhang M-Z, Bian C, Ye R-Z, et al. Human Infection with a Novel Tickborne Orthonairovirus Species in China. N Engl J Med [Internet]. **2025**; 392(2):200–202. Available from: https://www.ncbi.nlm.nih.gov/pubmed/39778175

10. Kodama F, Yamaguchi H, Park E, et al. A novel nairovirus associated with acute febrile illness in Hokkaido, Japan. Nat Commun. **2021**; 12(1):5539.

11. Lv X, Liu Z, Li L, et al. Yezo Virus Infection in Tick-Bitten Patient and Ticks, Northeastern China - Volume 29, Number 4—April 2023 - Emerging Infectious Diseases journal - CDC. Emerg Infect Dis. **2023**; 29(4):797–800.

12. Kosaka KI, Tsutomu S, Matsuno K. Yezo virus infection developed after a tick bite while climbing a stream in the Hidaka Mountains, Hokkaido, Japan. Japanese Journal of Mountain Medicine. **2024**; 1(43).

13. Suzuki K, Suzuki S, Yamaguchi H, Kakinoki Y. A Case of Tick-borne Disease with Yezo Virus and Borrelia miyamotoi Coinfection. Intern Med. **2024**; 63(20):3225–23.

14. Ogata Y, Sato T, Kato K, et al. A Case of Tick-borne Yezo Virus Infection: Concurrent Detection in the Patient and Tick. Int J Infect Dis. **2024**; 143(143):107038.

15. Morita K, Tachikawa N, Matsuda T, et al. A Case of Co-infection with Yezo Virus and Borrelia miyamotoi. J Hosp Gen Med. **2025**; 7(3):92–95.

16. Noguchi A, Komatsu S, Hashimoto Y, et al. A case of Yezo virus infection. Rinsho Hifuka (Japanese Journal of Clinical Dermatology). **2024**; 7(78):465–470.

17. Zhang M-Z, Bian C, Ye R-Z, et al. A series of patients infected with the emerging tick-borne Yezo virus in China: an active surveillance and genomic analysis. Lancet Infect Dis. **2025**; 25(4):390–398.

18. Chen Z-Y, Zhang J, He P-J, et al. Characteristics of viral ovarian tumor domain protease from two emerging orthonairoviruses and identification of Yezo virus human infections in northeastern China as early as 2012. J Virol. **2025**; 99(2):e01727-24.

19. Ito M, Minamikawa M, Kovba A, et al. Environmental and host factors underlying tick-borne virus infection in wild animals: Investigation of the emerging Yezo virus in Hokkaido, Japan. Ticks Tick Borne Dis. **2024**; 15(6):102419.

20. Su S, Cui MY, Xing LL, et al. Metatranscriptomic analysis reveals the diversity of RNA viruses in ticks in Inner Mongolia, China. PLoS Negl Trop Dis. **2024**; 18(12):e0012706.

21. Ni X-B, Cui X-M, Liu J-Y, et al. Metavirome of 31 tick species provides a compendium of 1,801 RNA virus genomes. Nat Microbiol. **2023**; 8(1):162-173.

22. Kartashov M, Svirin K, Zheleznova A, et al. First Report of the Yezo Virus Isolates Detection in Russia. Viruses. **2025**; 17(8):1125.

23. Nishino A, Tatemoto K, Ishijima K, et al. Transboundary Movement of Yezo Virus via Ticks on Migratory Birds, Japan, 2020-2021. Emerg Infect Dis. **2024**; 30(12):2674-2678.

24. Wang SS, Liu JY, Wang BY, et al. Geographical distribution of Ixodes persulcatus and associated pathogens: Analysis of integrated data from a China field survey and global published data. One Heal. **2023**; 16:100508.

25. Minh BQ, Schmidt HA, Chernomor O, et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol Biol Evol. **2020**; 37(5):1530-1534.

26. Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler A von, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. **2017**; 14(6):587-589.

27. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Syst Biol. **2010**; 59(3):307-321.

28. Hoang DT, Chernomor O, Haeseler A von, Minh BQ, Vinh LS. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol Biol Evol. **2018**; 35(2):518-522.

29. Bianchini G, Sánchez-Baracaldo P. TreeViewer: Flexible, modular software to visualise and manipulate phylogenetic trees. Ecol Evol. **2024**; 14(2):e10873.

30. Takahashi T, Maeda K, Suzuki T, et al. The First Identification and Retrospective Study of Severe Fever With Thrombocytopenia Syndrome in Japan. J Infect Dis. **2014**; 209(6):816–827.

31. Jevtic D, Silva MD da, Haylock AB, et al. Hemophagocytic Lymphohistiocytosis (HLH) in Patients with Tick-Borne Illness: A Scoping Review of 98 Cases. Infect Dis Rep. **2024**; 16(2):154–169.

32. Kim UJ, Oh T hoon, Kim B, et al. Hyperferritinemia as a Diagnostic Marker for Severe Fever with Thrombocytopenia Syndrome. Disease Markers [Internet]. **2017**; 2017(1):6727184. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1155/2017/6727184

Figure Legends

Figure 1. Flow chart of surveillance for Yezo virus infections

Retrospective (n = 388, 2013–2022) and prospective (n = 163, 2023–2024) surveillance for Yezo virus infections were conducted using serum samples collected from patients suspected to tick-bore febrile illness. Following the specific antibody detections (IgM and IgG ELISA), RNA was extracted and subjected to the genetic detection (RT-qPCR).

Figure 2. Geographic and temporal distributions of Yezo virus-positive cases identified in Hokkaido, Japan

A) The location of Hokkaido is shown in the map of Far Eastern Asia. Orange-colored area surrounded by a dashed line indicates the current potential endemic area of Yezo virus infections. Hokkaido has been divided into four regions based on the local administration areas. B) Annual and regional distribution of Yezo virus-positive cases identified through retrospective and prospective surveillance conducted between 2013 and 2024. Each region in Hokkaido is colored identically in the map and graph. C) Monthly distribution of disease onset dates among patients with confirmed Yezo virus infection. 20 cases for which the onset dates were available, are shown. D) Incubation period of Yezo virus infections. Each dot represents the incubation period (days) of a patient based on the duration between the reported date of tick bite and the date when any symptoms were firstly recognized.

Figure 3. Temporal profile of viral RNA and antibody detection in Yezo virus-infected patients

A) Results of viral RNA detection by RT-qPCR, and specific antibody detection by IgM and IgG ELISA, and neutralizing antibody detection by focus reduction neutralization test. Each dot represents one serum sample. A total of 42 serum samples from 17 YEZV-infected patients with recorded disease onset dates were used. B) Seroconversion profiles of eight patients whose paired sera were available. C) Neutralizing antibody titers in one Yezo virus-infected patient, followed for over 600 days post disease onset.

Figure 4. Phylogenetic analysis among Yezo viruses

Phylogenetic trees based on A) nucleoprotein gene on S segment, B) glycoprotein precursor gene on M segment, and C) RNA-dependent RNA polymerase gene on L segment of Yezo viruses were constructed by using maximum likelihood inference. The outgroup (Sulina virus) of the S segment tree was removed from each tree for visualization of the phylogenetic relationships among Yezo virus strains. Strain names are indicated with the tips and labeled with their origins (countries), genetic clades, and potential genetic reassortment.

Table1. Summary of YEZV surveillance between 2013 and 2024

Methods	Retrospective (2013–2022, n=388)	Prospective (2023–2024, n=163)	Total
ELISA	11/388 (2.8%)	8/163 (4.9%)	19/551 (3.4%)
RT-qPCR	11/388 (2.8%)	7/163 (4.3%)	18/551 (3.3%)
ELISA+RT-qPCR	8/388 (2.1%)	7/163 (4.3%)	15/551 (2.7%)
Total	14/388 (3.6%)	8/163 (4.9%)	22/551 (4.0%)

Table 2. Basic characteristics of Yezo virus infections in Japan

	n=20
Sex	
Male	15 (75.0%)
Female	5 (25.0%)
Age	
Median [IQR]	59.5 [40.3-71.5]
0-19	0 (0.0%)
20-29	2 (10.0%)
30-39	3 (15.0%)
40-49	3 (15.0%)
50-59	2 (10.0%)
60-69	4 (20.0%)
70-79	4 (20.0%)
80-89	2 (10.0%)
90-	0 (0.0%)
Underlying medical condition (≥2)	
Hypertension	3 (15.0%)
Dyslipidemia	3 (15.0%)
Tick bite within 2 weeks of onset	18 (90.0%)

Table 3. Clinical characteristics of Yezo virus infections in Japan

Clinical condition	positive/total
Febrile illness	
Fever	19/20 (95.0%)
Fever >38℃	10/14 (71.4%)
General symptoms	
General fatigue	10/20 (50.0%)
Myalgia/Arthralgia	10/20 (50.0%)
Headache	12/20 (60.0%)
Gastrointestinal tract symptoms	
Overall	10/20 (50.0%)
Nausea	2/20 (10.0%)
Vomiting	0/20 (0.0%)
Abdominal pain	3/20 (15.0%)
Diarrhea	2/20 (10.0%)
Anorexia	7/20 (35.0%)
Respiratory symptoms	
Overall	0/20 (0.0%)
Throat pain	0/20 (0.0%)
Cough	0/20 (0.0%)
Others	
Lymphadenopathy	1/20 (5.0%)
Laboratory finding	
Leukopenia	19/20 (95.0%)
Thrombocytopenia	20/20 (100%)
Liver dysfunction	17/20 (85.0%)
Serum chemistry	
Total protein level < 6.0 mg/dL (hypoproteinemia)	0/7 (0.0%)
Albumin level < 3.0 mg/dL (hypoalbuminemia)	0/7 (0.0%)
Aspartate aminotransferase level >30 IU/L	14/14 (100%)
Alanine aminotransferase level >30 IU/L	12/14 (85.7%)
Lactate dehydrogenase level >250 IU/L	8/11 (72.7%)
Creatine kinase level >200 IU/L	3/9 (33.3%)
Blood urea nitrogen level >20 mg/dL	0/7 (0.0%)
Creatinine level >1 mg/dL	3/7 (42.9%)
C-reactive protein level >1 mg/dL	2/11 (18.2%)
Increased ferritin level	7/7 (100%)

Figure 1

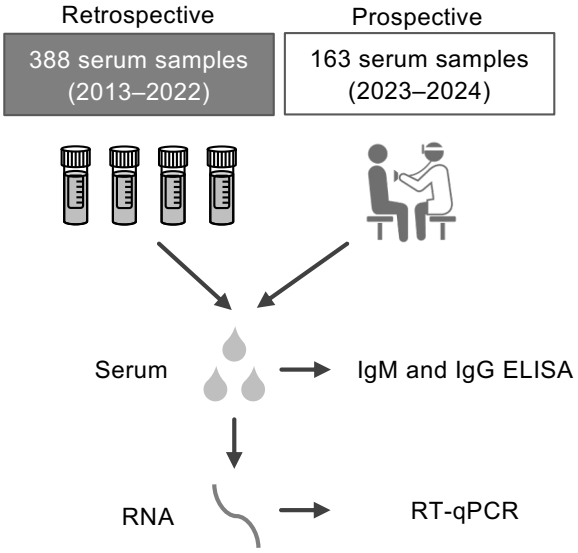

Retrospective
388 serum samples
(2013–2022)
Prospective
163 serum samples
(2023–2024)
Serum
IgM and IgG ELISA
RNA
RT-qPCR

Figure 2

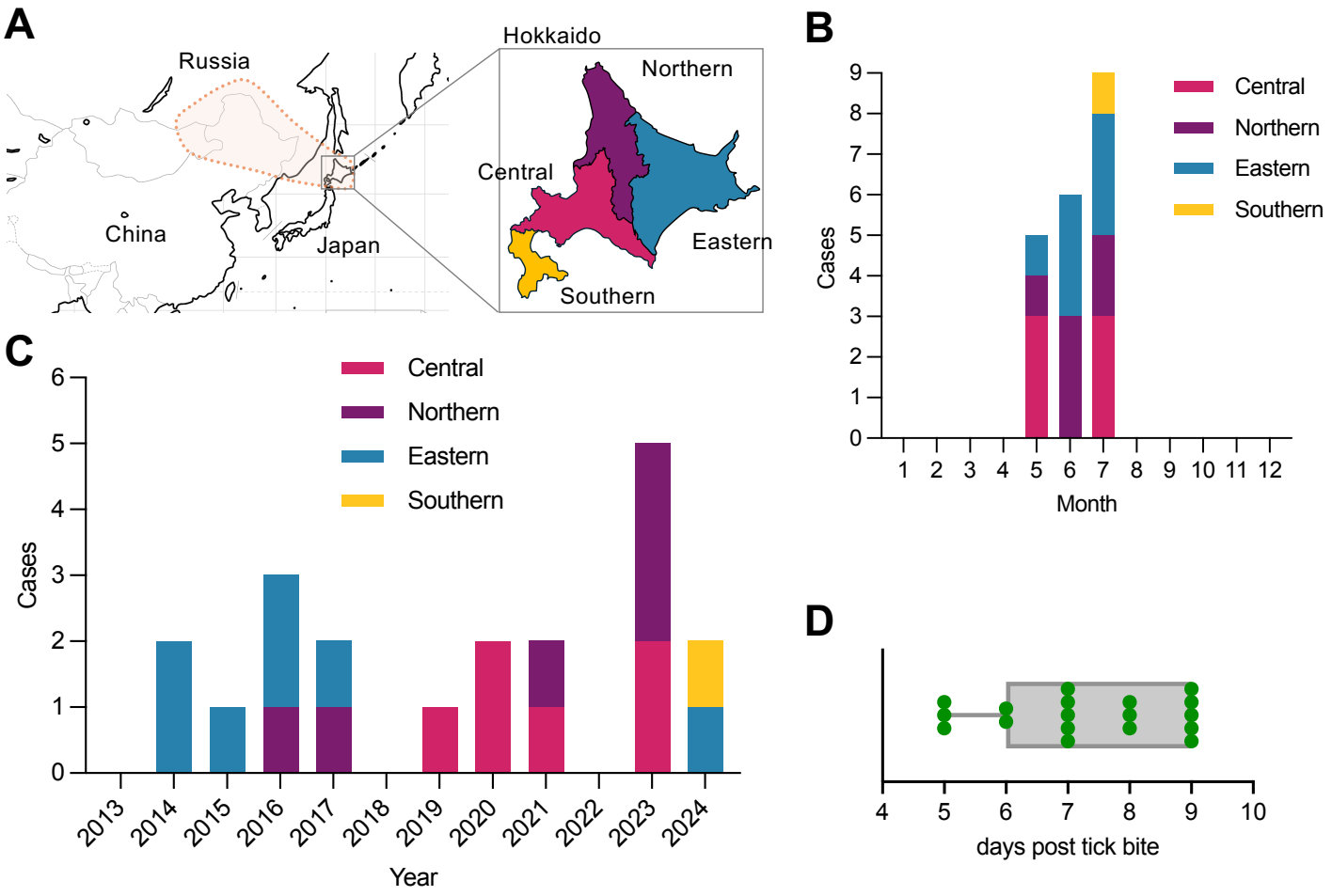

A
Russia
China
Japan
Hokkaido
Northern
Central
Eastern
Southern
B
Central
Northern
Eastern
Southern
Cases
Month
C
Central
Northern
Eastern
Southern
Cases
Year
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
D
days post tick bite

Figure 3

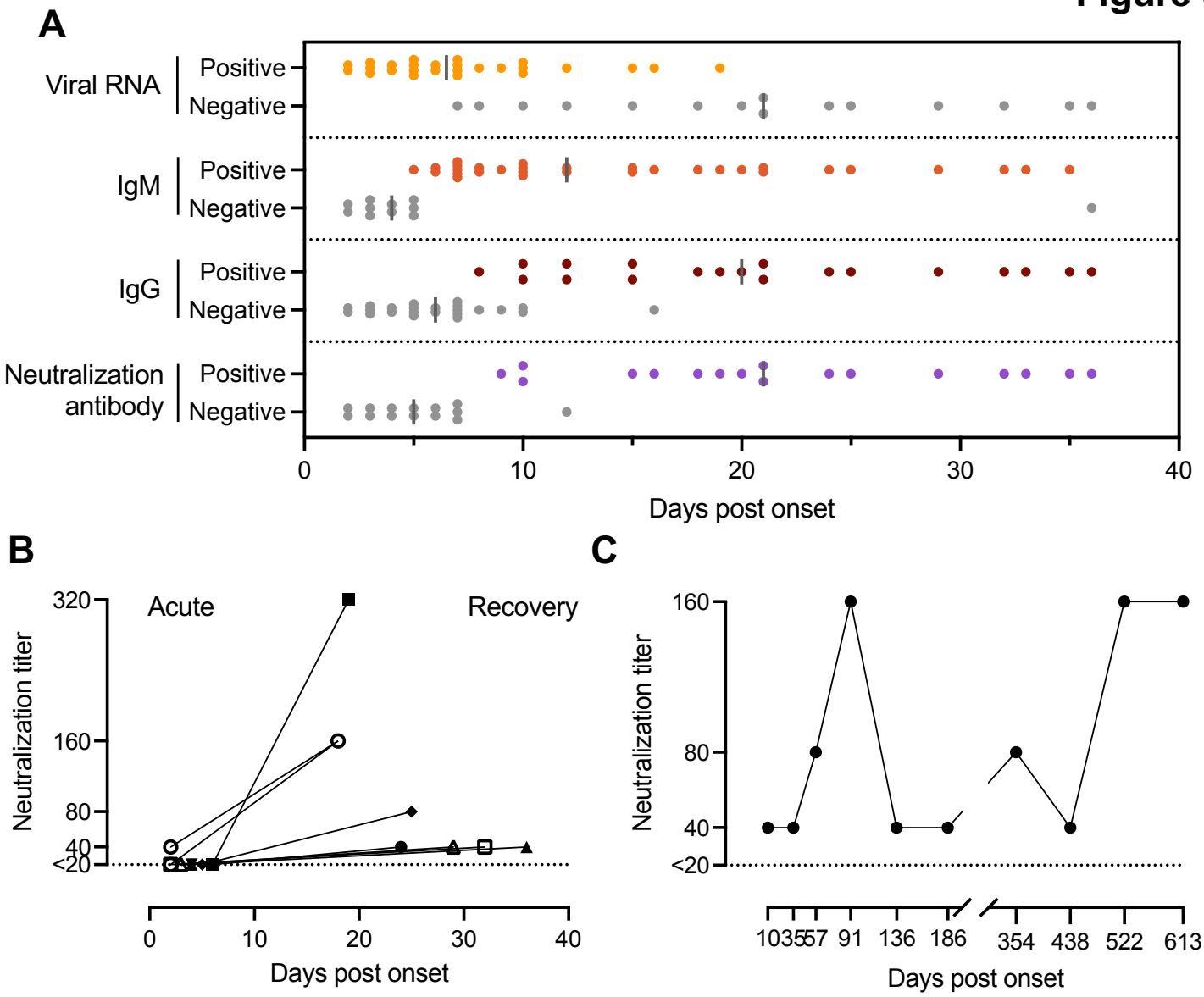
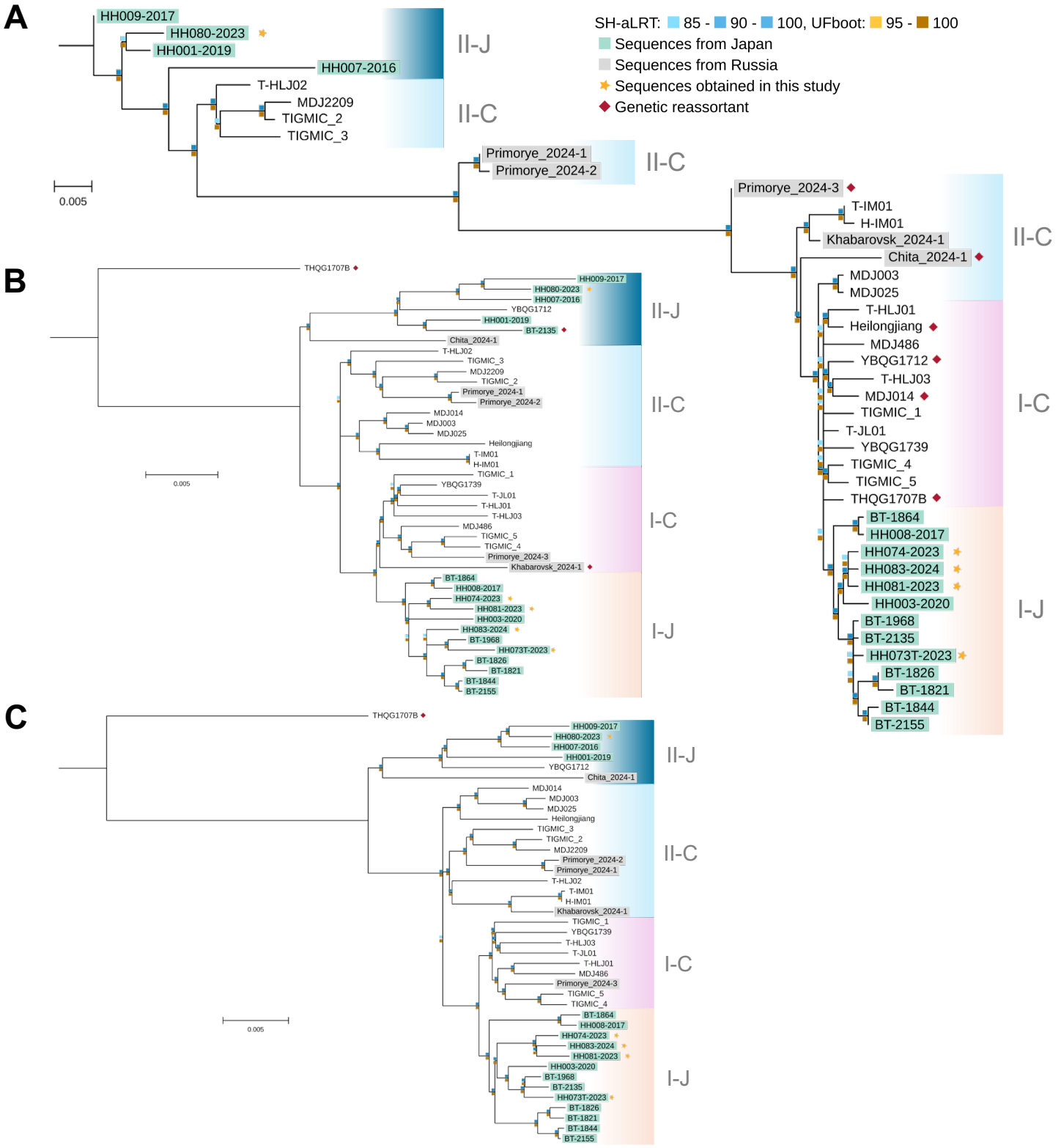

A
Viral RNA
Positive
Negative
IgM
Positive
Negative
IgG
Positive
Negative
Neutralization antibody
Positive
Negative
0
10
20
30
40
Days post onset
B
320
160
80
40
<20
Neutralization titer
Acute
Recovery
0
10
20
30
40
Days post onset
C
160
80
40
<20
Neutralization titer
10
35
57
91
136
186
354
438
522
613
Days post onset

Figure 4


A
B
C
SH-aLRT: 85 - 90 - 100, UFboot: 95 - 100
Sequences from Japan
Sequences from Russia
Sequences obtained in this study
Genetic reassortant
II-J
II-C
I-C
I-J
0.005
HH009-2017
HH080-2023
HH001-2019
HH007-2016
T-HLJ02
MDJ2209
TIGMIC_2
TIGMIC_3
Primorye_2024-1
Primorye_2024-2
Primorye_2024-3
T-IM01
H-IM01
Khabarovsk_2024-1
Chita_2024-1
MDJ003
MDJ025
T-HLJ01
Heilongjiang
MDJ486
YBQG1712
T-HLJ03
MDJ014
TIGMIC_1
T-JL01
YBQG1739
TIGMIC_4
TIGMIC_5
THQG1707B
BT-1864
HH008-2017
HH074-2023
HH083-2024
HH081-2023
HH003-2020
BT-1968
BT-2135
HH073T-2023
BT-1826
BT-1821
BT-1844
BT-2155