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# Detection of Yezo virus (*Nairoviridae, Orthonairovirus, Orthonairovirus yezoense*) circulation in *Ixodes persulcatus* ticks in the Amur Region, Russia

Mikhail Yu. Kartashov✉, Valentina Yu. Kurushina, Alina S. Zheleznova, Kirill A. Svirin, Ekaterina I. Materikina, Vladimir A. Ternovoi

State Research Center of Virology and Biotechnology «Vector», 630559, Koltsovo, Novosibirsk region, Russia

**Abstract**

**Introduction.** The unfavorable epidemiological situation with viral tick-borne infections can be characterized not only by an increase in the incidence of «classical», well-known diseases, but also by the discovery of new pathogens, the role of which remains poorly understood. *Yezo virus* (YEZV) belongs to the group of recently discovered tick-borne nairoviruses. YEZV circulation is characteristic of Japan, China, and a number of regions in Russia (Primorsky, Khabarovsk, and Zabaykalsky Krais; Tomsk Oblast).

The **aim** of the study was to screen and characterize the molecular genetics of YEZV isolated from ticks in the Amur region.

**Materials and methods.** In the study, 704 individual tick samples (463 *I. persulcatus* ticks and 241 *H. concinna* ticks) collected from vegetation in 15 locations in 5 districts of the Amur Region were analyzed for the presence of YEZV RNA. Full-length genomic sequencing of the identified YEZV genetic variant was performed by preliminary enrichment of YEZV cDNA using multiplex PCR with a primer panel developed by us.

**Results.** The YEZV infection rate in the studied sample of *I. persulcatus* ticks was 0.2% (1/463; 95% CI: 0.1–1.2).

**Conclusion.** The results of this study expand our understanding of the spread of YEZV in Russia. Up-to-date information on the circulation and genetic diversity of nairoviruses that pose a potential threat to humans and animals contributes to the regulation and adjustment of preventive and anti-epidemic measures, as well as the implementation of adequate region-oriented laboratory diagnostics of IPK in the Far East, and especially in areas bordering China.

**Keywords:** *Orthonairovirus; Yezo virus; YEZV; ixodid ticks; Amur Region*

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**Conflict of interest.** The authors declare no apparent or potential conflicts of interest related to the publication of this article.

**ОРИГИНАЛЬНЫЕ ИССЛЕДОВАНИЯ**
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# Выявление циркуляции Yezo virus (*Nairoviridae*, *Orthonairovirus*, *Orthonairovirus yezoense*) в клещах *Ixodes persulcatus* на территории Амурской области

Карташов М.Ю.✉, Курушина В.Ю., Железнова А.С., Свирин К.А., Материкина Е.И., Терновой В.А.

ФБУН «Государственный научный центр вирусологии и биотехнологии «Вектор» Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека (Роспотребнадзор), 630559, р.п. Кольцово, Новосибирская область, Россия

**Резюме**

**Введение.** Неблагополучная эпидемиологическая ситуация с вирусными инфекциями, переносимыми клещами (ИПК), может характеризоваться не только ростом встречаемости уже известных заболеваний, но и выявлением новых возбудителей, роль которых пока остается малоизученной. К группе недавно открытых наировирусов, переносимых клещами, относится *Yezo virus* (YEZV). Циркуляция YEZV характерна для Японии, Китая и ряда регионов России (Приморский, Хабаровский, Забайкальский края, Томская область).

**Цель** работысостояла в доказательстве циркуляции на территории Амурской области недавно открытого наировируса - YEZV, а также его молекулярно-генетической характеристике путем определения полногеномной нуклеотидной последовательности выявленного генетического варианта YEZV.

**Материалы и методы.** В исследовании на наличие РНК YEZV проанализированы 704 индивидуальные пробы клещей (463 клеща вида *Ixodes persulcatus* и 241 клещ вида *Haemaphysalis concinna*), отловленных с растительности в 15 локациях, расположенных в 5 районах Амурской области. Проведено секвенирование полноразмерной геномной последовательности выявленного генетического варианта YEZV путем предварительного обогащения кДНК YEZV методом мультиплексной полимеразной цепной реакции с использованием разработанной нами панели праймеров.

**Результаты.** Уровень инфицированности YEZV в исследуемой выборке клещей *I. persulcatus* составил 0,2% (1/463; 95% ДИ 0,1–1,2).

**Заключение.** Полученные данные расширяют представления о распространении YEZV на территории России. Актуальная информация о циркуляции и генетическом разнообразии наировирусов, представляющих потенциальную опасность для человека и животных, способствует регулированию и корректировке профилактических и противоэпидемических мероприятий, а также проведению адекватной регионально-ориентированной лабораторной диагностики ИПК на территории Дальнего Востока, и прежде всего в приграничных с Китаем областях.

**Ключевые слова:** *наировирусы; вирус Йезо; YEZV; иксодовые клещи; Амурская область*

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**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

## Introduction

Tick-borne diseases (TBDs) remain one of the most significant problems of infectious pathology in a significant part of the Russian Federation. According to official statistics, the number of people seeking medical help in the Russian Federation for tick bites in 2024 was 314.86 per 100,000 population (which in absolute terms amounts to approximately 500,000 cases). It should also be noted that not all victims seek medical attention for tick bites and are included in the statistics, so the actual number of such cases may be significantly higher.

The epidemiological significance of TBDs is determined not only by the high incidence of well-known nosological forms (which primarily include viral tick-borne encephalitis, ixodid tick-borne borreliosis, tick-borne rickettsiosis), but also by the constant addition of new pathogens (primarily viral in nature) to the list, whose role in infectious pathology remains poorly understood. Metagenomic analysis approaches based on modern methods of mass parallel sequencing have made it possible to identify many new viruses carried by ticks and causing disease in humans. These newly discovered viruses include representatives of flavivirus-like viruses with segmented genome (*Jingmen tick virus, Alongshan tick virus, Yanggou tick virus*, etc.), *Bourbon virus* and *Dabie bandavirus* (formerly known as SFTS virus) [1–3]. A significant number of viruses transmitted by ticks and capable of causing disease in humans and mammals with a wide range of clinical manifestations belong to the genus *Orthonairovirus* of the family *Nairoviridae* [4]. Representatives of this taxonomic group include both well-known pathogens (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus, Dugbe virus) and recently discovered viruses (*Tacheng tick-borne 1 virus, Songling virus, Beiji nairovirus*, etc.). Yezo virus (YEZV) can also be included in the group of recently discovered tick-borne nairoviruses. YEZV was first detected in Japan (on the island of Hokkaido) in two patients with fever, leukopenia, and thrombocytopenia that appeared after a tick bite. Retrospective studies of serum samples from 248 patients living on Hokkaido Island with a history of fever of unknown etiology that developed after a tick bite revealed YEZV RNA in five more people [5]. Studies of ticks inhabiting Hokkaido Island have detected YEZV genetic material in three species of ticks: *Ixodes persulcatus*, *I. ovatus* and *Haemaphysalis megaspinosa*, with a prevalence ranging from 1.3% to 3.7%. Serological studies have shown that YEZV infection is also common among wild animals. For example, on Hokkaido Island, antibodies to YEZV were detected in serum samples from raccoons (*Procyon lotor*) and raccoon dogs (*Nyctereutes procyonoides albus*) [5].

YEZV RNA was detected in an extensive study of ticks collected in northeastern China (Inner Mongolia, Heilongjiang, and Jilin provinces), where the infection rate in *I. persulcatus* ticks was 0.4–0.5%. YEZV infection has also been reported in China. In a study involving 402 patients with a history of tick bites, YEZV RNA was detected in one subject [6].

In Russia, YEZV genetic material was detected in *I. persulcatus* ticks collected in Primorsky, Khabarovsk, and Zabaykalsky Krais [7], as well as in *I. pavlovskiy* ticks inhabiting the Tomsk Region [8].

The Amur region, one of the key industrial and agricultural regions of the Far East, is characterized by a high incidence of tick bites among the population and an increase in the intensity of the epidemic process of TBDs, recorded over the past few years [9]. To date, only three nosological forms of TBDs are officially registered in the Amur Region: viral tick-borne encephalitis, ixodid tick-borne borreliosis, and tick-borne rickettsiosis. However, as mentioned above, the tense epidemiological situation with regard to TBDs may be determined not only by the increase in the incidence of already known nosological forms, but also by the discovery of new pathogens and the infections they cause, whose role in regional infectious pathology remains little or virtually unexplored. The geographical proximity of the Amur Region to the interior regions of China, where a wide variety of pathogenic viruses, including YEZV, have been reported [10, 11], as well as the widespread distribution of the arthropod vectors themselves and the constant expansion of areas subject to anthropogenic impact, make it necessary to monitor the entire known spectrum of tick-borne pathogens in this territory.

**The aim** of the study was to prove the circulation of the recently discovered YEZV nairovirus in the Amur Region, as well as to characterize its molecular genetics by determining the whole-genome nucleotide sequence of the identified YEZV genetic variant.

## Materials and methods

A total of 463 individual samples of *I. persulcatus* ticks (250 females and 213 males) and 241 individual samples of *H. concinna* ticks (132 females and 109 males) were collected and analyzed in the study. The ticks were collected from vegetation using the "flag" method in 15 locations in five districts of the Amur Region (Arkharinsky, Bureysky, Svobodnensky, Skovorodinsky and Shimanovsky) (**Fig. 1**). This stage of the study was carried out in compliance with the biosafety rules regulated by MU 1.3.2569-09 and SanPiN 3.3686-21. The coordinates of the tick collection sites were recorded using a Garmin GPSMAP 65s GPS navigator (USA). A map showing the locations where the ticks were collected was created using the online service uMap (https://umap.openstreetmap.fr/ru/) and a free map layer from OpenStreetMap (https://www.openstreetmap.org/) (Fig. 1). Before starting molecular genetic testing of ticks, they were washed twice with 70% ethanol to remove external contaminants and external microflora.

The samples of the studied ticks were mechanically homogenized in 300 μl of sterile phosphate-buffered saline using a TissueLyser LT homogenizer (Qiagen, Germany). Total nucleic acids were extracted using the phenol-chloroform extraction method with the LIRA+ reagent kit (Biolabmix, Russia). The first strand of complementary DNA (cDNA) was synthesized in a reverse transcription reaction using MMLV reverse transcriptase and random decanucleotide primers (Eurogen, Russia). The obtained samples were tested for the presence of YEZV RNA using real-time polymerase chain reaction (PCR) with a pair of primers (YEZV_F: CACCAGGCATTTACCTCTACTT and YEZV_R: TGGAGTCAAGGGCTGTTATG) and a fluorescently labeled probe (YEZV_Z: CY5-TGCCAGGGCTACTGTGATGCATAA-BQ2), corresponding to the M segment fragment. Real-time PCR amplification and detection were performed on a BioRad CFX-96 thermocycler (Bio-Rad, USA) in a 25 μL reaction mixture prepared using the BioMaster HS-qPCR kit (2×) (Biolabmix, Russia). Real-time PCR results were recorded according to the standard method based on the presence or absence of intersection of the fluorescence curve with the established threshold line. Fluorescence signal accumulation curves were analyzed using BioRad CFX-96 software; the criterion for a positive sample at the screening stage was a typical sigmoid shape of the signal accumulation curve and a Ct value not exceeding 35.

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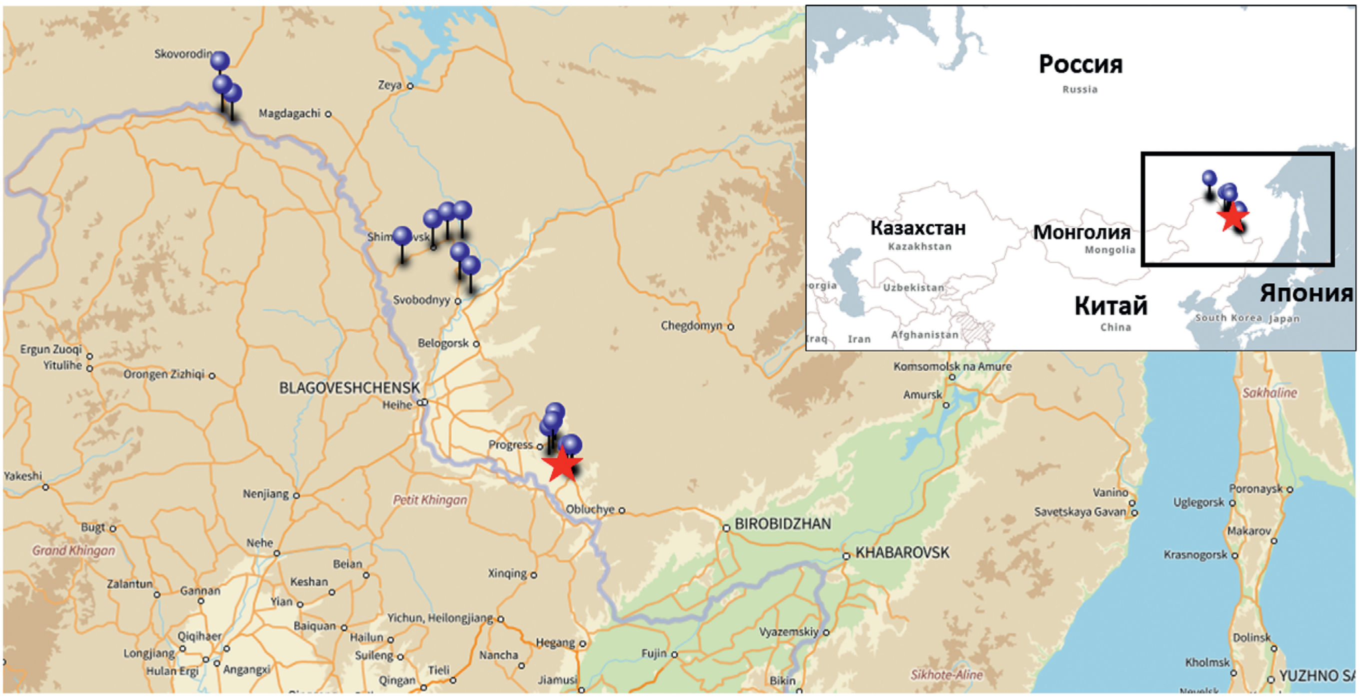


**Fig. 1.** Locations where the studied ticks were collected (marked with blue markers).
The location where YEZV RNA was detected in the collected ticks is marked in red.

**Рис. 1.** Места отлова исследуемых клещей (отмечены синими метками).
Красным отмечена локация, где в собранных клещах была выявлена РНК YEZV.

To identify genetic markers of other TBD pathogens (tick-borne encephalitis virus, *Borrelia burgdorferi* s.l., *B. miyamotoi*, *Ehrlichia chaffeensis*, *E. muris*, *Anaplasma phagocytophilum*), "Amplisens TBEV, B. burgdorferi sl, A. phagocytophillum, E. chaffeensis/E. muris-FL. Detection of infectious agents transmitted by ixodid ticks" (RU: FSR 2010/09026) and "AmpliSens Borrelia miyamotoi-FL. Detection of Borrelia miyamotoi" (RU: RZN 2018/7316) (AmpliSens, Russia) commercially available kits were used. To detect Rickettsia DNA, a pair of primers were used (PrF_gltA: GGCTTCGGTCATCGTGT and PrR_gltA: TTGCTATTTGTAAGAGCGGATTG), as well as an oligonucleotide probe Z(ROX)_gltA: ROX-CCACGTGCCGCAGTACTTAAAGAAAC-BHQ2' [12]. Real-time PCR was performed on a BioRad CFX-96 thermocycler (Bio-Rad, USA) according to the manufacturer's instructions or the recommended protocol for published primers.

To perform whole-genome sequencing of the identified YEZV genetic variant, cDNA enrichment was first carried out using multiplex PCR with a primer panel developed by us, which allows amplification of overlapping fragments with an average length of about 1000 nucleotides (the length of the fragment overlap region is about 150 nucleotides) [7]. The primers included in this panel were selected using the PerlPrimer v. 1.1.21 program [13] based on degenerate consensus sequences obtained by aligning the available full-length sequences of the S, M and L segments of YEZV. The optimal annealing temperature of oligonucleotides and the probability of specific interactions between primers were determined using the OligoCalc service [14]. Multiplex enrichment was performed in 4 reaction mixtures using the BioMaster HS-Tag PCR kit (Biolabmix, Russia), followed by evaluation of enrichment efficiency by electrophoresis in 2% agarose gel and measurement of double-stranded DNA concentration on a Qubit 2.0 device using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, USA).

Library preparation for high-throughput sequencing was performed using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB, UK). The preparation process included fragmentation, end repair, and dA-tailing, as well as adapter ligation using a single enzyme mixture. Sequencing was performed on the Illumina MiSeq platform (Illumina, USA). To remove adapters, short sequences, and low-quality sequences (quality score less than 20 and length less than 30 nucleotides), FASTQ files were processed using fastp v. 0.20.1 (https://github.com/OpenGene/fastp/) [15]. Pre-processed reads were aligned to the reference genome YEZV (LC790676, LC790675, LC790674) obtained from the NCBI GenBank database using BWA MEM v. 0.7.18 (https://github.com/lh3/bwa) [16]. SAM/BAM files were processed and analyzed using Samtools v. 1.11 [17]. The iVar v. 1.2.2 program [18] was used to extract the consensus sequence from BAM files.

Phylogenetic trees were constructed in MEGA 11 [19] using the maximum likelihood method with the Tamura–Nei evolutionary model (TN93). The statistical significance of the topology of phylogenetic trees was assessed using the Bootstrap analysis method with 1000 pseudo-samples. Visualization was performed using the iTOL program [20].

When performing statistical analysis of the data and calculating the 95% confidence interval (CI), Wilson's estimate without continuity correction (https://pedro.org.au/wp-content/uploads/CIcalculator.xls) was used.

## Results

When screening 463 samples of *I. persulcatus* ticks for the presence of YEZV RNA, one sample containing YEZV genetic material (Ct = 24.3) was identified. The YEZV infection rate in the studied sample of *I. persulcatus* ticks was 0.2% (1/463; 95% CI 0.1–1.2). The YEZV-infected tick was caught in the Arkharinsky District near the Amur federal highway (geographical coordinates: 49.468778; 130.158701). No YEZV RNA was detected in the studied sample of *H. concinna* ticks. For the identified genetic variant of YEZV, full-length sequences of all three genome segments were determined and deposited in the GenBank database under numbers PX353463 for segment S, PX353464 for segment M, and PX353465 for segment L.

Phylogenetic analysis did not show clear clustering of known YEZV isolates by geographical location (prototype variants isolated in China and Japan) or source of isolation (prototype variants isolated from ticks or humans).

Phylogenetic trees demonstrate that the YEZV genetic variant detected in the *I. persulcatus* tick in the Amur Region, when analyzed across all three segments, clearly clusters with the prototype YEZV sequences previously detected in China (the highest level of homology when compared by segments S and M is demonstrated with the YBQG1712 variant found in ticks from the Chinese province of Yanbian, and when compared by segment L with the Chinese variants MDJ003, MDJ014, MDJ025, isolated from patients in Heilongjiang Province, China). It is interesting to note that there is no clear clustering of the YEZV genetic variant from the Amur region with other variants circulating in Russia. Overall, when analyzing all three segments, the closest to the identified variant from Russian isolates is YEZV, found in the Khabarovsk region (**Fig. 2**).

The genetic similarity of nucleotide and deduced amino acid sequences between the YEZV variant detected in the Amur Region and a number of prototype YEZV isolates circulating in Russia, China, and Japan is shown in the **Table**. As expected, when compared with known prototypes, the most conservative segment in terms of both nucleotide and amino acid sequence is segment L, which encodes RNA-dependent RNA polymerase.

The highest level of intraspecies variation in the nucleotide sequences of YEZV genetic variants found in Russia (analysis performed for the

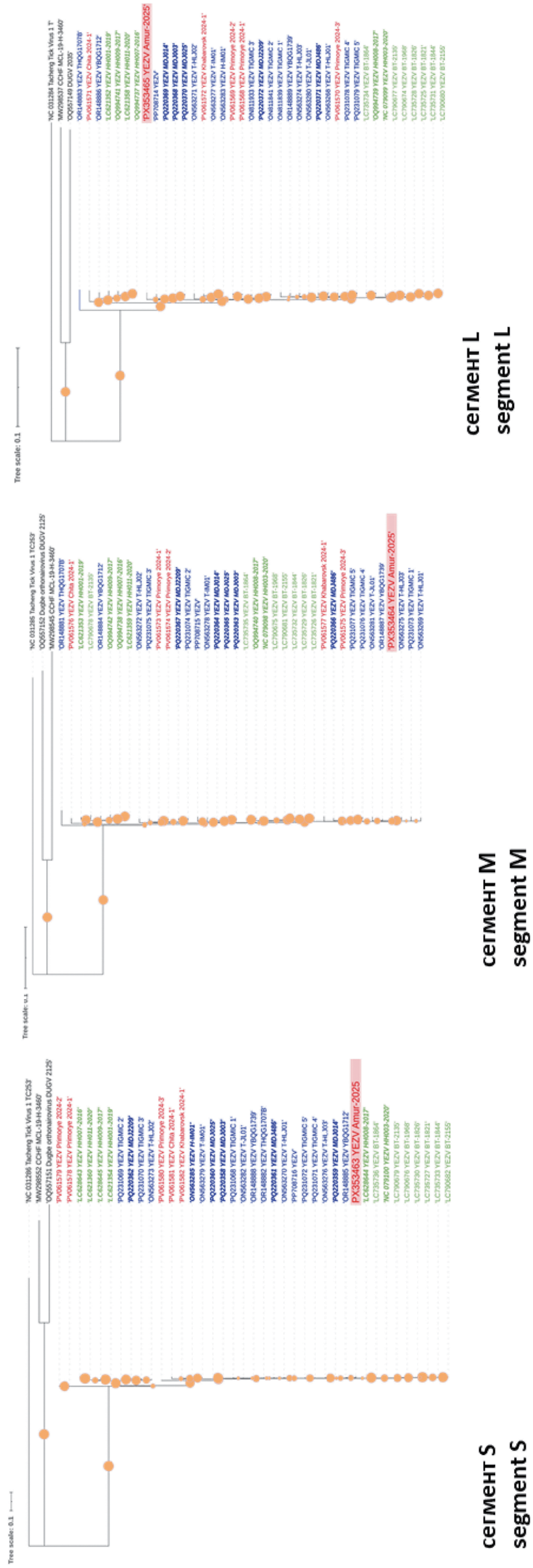


**Fig. 2.** Phylogenetic trees constructed by analyzing full-length sequences of YEZV segments.
Isolates circulating in Russia are highlighted in red; isolates circulating in China are highlighted in blue; isolates circulating in Japan are highlighted in green.

**Рис. 2.** Филогенетические деревья, построенные при анализе полноразмерных последовательностей сегментов YEZV.
Красным шрифтом выделены варианты, обнаруженные в России; синим цветом выделены варианты, обнаруженные в Китае; зеленым цветом выделены варианты, обнаруженные в Японии.

identified genetic variant and previously identified Primorye-2024-1, Primorye-2024-2, Primorye-2024-3, Chita-2024-1 and Khabarovsk-2024-1) is characteristic of segment S (the average difference when comparing full-length sequences is 2.8%). The differences in nucleotide sequences for the M and L segments are 1.7%. When comparing the deduced amino acid sequences, variability of these segments ranges from 0.3% (for the L segment) to 0.5% (for the S and M segments) (**Fig. 3**).

## Discussion

The presence of natural foci of various TBDs, the constant expansion of areas subject to anthropogenic impact, and the existence of a professional contingent working in natural foci areas determine the continued risk of infection with various human TBD pathogens in the Amur Region. According to official statistics, from 2014 to 2020, there was a downward trend in the incidence of TBDs in the Amur Region, but since 2021, there has been an upward trend in the intensity of the epidemic process from 0.26 to $2.08^0/_{0000}$ in 2023. [9]. The key result of this study is the first detection of YEZV RNA in *I. persulcatus* ticks in the Amur Region. The fact that YEZV genetic material has been detected in ticks in the Amur Region may indicate that, in addition to the officially registered nosological forms, the actual spectrum of TBDs in this territory may be much broader. In this regard, special attention should be paid to areas bordering China and territories that have been subjected to significant anthropogenic impact, as well as to areas remote from the regional center with lower levels of access to medical care, including laboratory diagnostics. It is interesting to study the immune response to YEZV living in this territory, as well as to identify molecular genetic and serological markers of YEZV among various mammals that are potential reservoirs of the virus.

An important aspect of studying the diversity and evolution of viruses is the study of the genetic stability of their genomes. It is known that viruses with a segmented genome quite often undergo rearrangement/recombination events, which contribute significantly to their genetic variability. However, the topology of phylogenetic trees for known genetic variants of YEZV, constructed for all three segments, is virtually identical, suggesting a high degree of adaptation of this virus to both invertebrate vectors and vertebrate hosts.

To rule out the possible integration of fragments of the YEZV viral genome into the tick genome [21, 22], PCR was performed in parallel with a positive sample without the reverse transcription step, which resulted in no amplicons being produced.

Natural foci of many TBDs, due to common vectors and reservoirs of infection, are usually combined, which raises the issue of the development of mixed infections. In this study, all samples of the ticks studied were additionally analyzed by PCR for the presence of genetic markers of tick-borne encephalitis virus, rickettsia, *B. burgdorferi* s.l., *B. miyamotoi*, and *A. phagocytophilum*. Among the sample of *I. persulcatus* ticks studied, no tick-borne encephalitis virus RNA was detected, and the infection rate of *B. burgdorferi* s.l. was 7.6% (35/463; 95% CI 5.5–10.3), *B. miyamotoi* – 4.1% (19/463; 95% CI 2.6–6.3), *A. phagocytophilum* – 1.9% (9/463; 95% CI 1.1–3.6). The highest infection rate was characteristic of rickettsiae, which were detected in 82.3% of the *I. persulcatus* ticks studied (381/463; 95% CI 78.5–85.5). Rickettsia DNA was also detected in 10.4% of *H.*

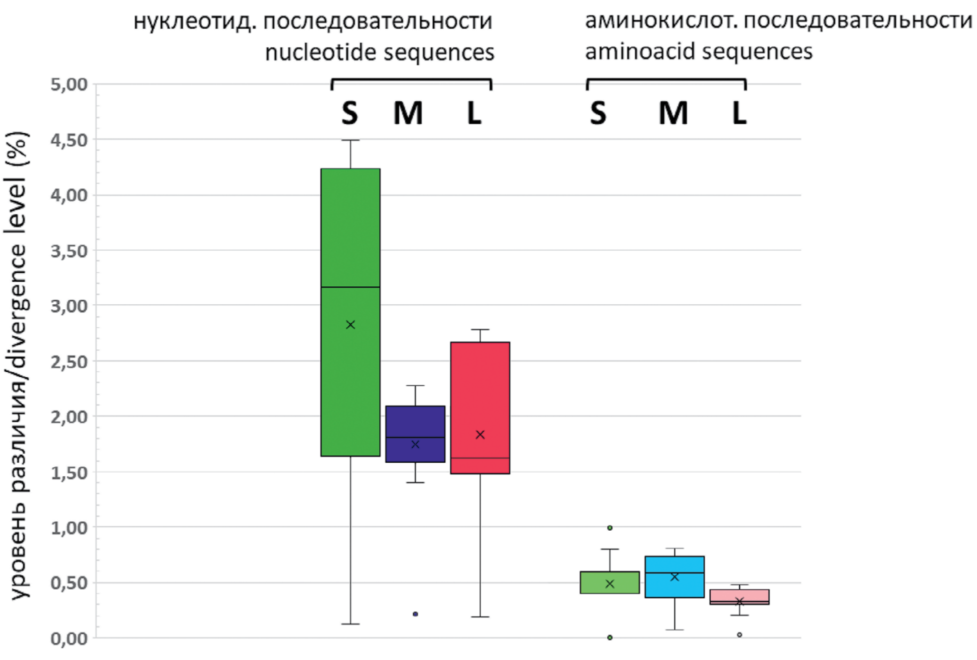


**Fig. 3.** Divergence between YEZV genetic variants circulating in Russia at nucleotide and amino acid level.

**Рис 3.** Уровень различия нуклеотидных и аминокислотных последовательностей генетических вариантов YEZV, циркулирующих на территории России.

**Table.** Level of identity of nucleotide and amino acid sequences (%) of YEZV isolates detected in the Amur Region compared to prototype isolates

**Таблица.** Уровень сходства нуклеотидной и аминокислотной последовательностей (%) выявленного в Амурской области варианта YEZV по сравнению с прототипными изолятами

| Prototype isolate (location and source of isolation)<br>Прототипный вариант (место и источник обнаружения) | Segment S<br>Сегмент S | | Segment M<br>Сегмент M | | Segment L<br>Сегмент L | |
|---|---|---|---|---|---|---|
| | nuc | aa | nuc | aa | nuc | aa |
| Primorye-2024-1 (Russia, Primorsky Krai, tick)<br>(Россия, Приморский край)<br>PV061578, PV061573, PV061568 | 95.51 | 99.40 | 98.12 | 99.48 | 98.37 | 99.67 |
| Primorye-2024-2 (Russia, Primorsky Krai, tick)<br>(Россия, Приморский край)<br>PV061579, PV061574, PV061569 | 95.64 | 99.40 | 98.15 | 99.41 | 98.34 | 99.70 |
| Primorye-2024-3 (Russia, Primorsky Krai, tick)<br>(Россия, Приморский край)<br>PV061580, PV061575, PV061570 | 97.98 | 99.40 | 98.60 | 99.71 | 98.23 | 99.75 |
| Chita-2024-1 (Russia, Transbaikal Region, tick)<br>(Россия, Забайкальский край)<br>PV061581, PV061576, PV061571 | 97.98 | 99.00 | 97.77 | 99.19 | 98.51 | 99.60 |
| Khabarovsk-2024-1 (Russia, Khabarovsk Krai, tick)<br>(Россия, Хабаровский край)<br>PV061582, PV061577, PV061572 | 98.36 | 99.40 | 98.41 | 99.49 | 98.50 | 99.79 |
| YBQC1712 (China, Yanbian, tick)<br>(Китай, Yanbian, клещ)<br>OR148885, OR148884, OR148886 | 98.55 | 99.40 | 97.41 | 99.34 | 97.41 | 99.75 |
| THQC1707B (China, Tonghua, tick)<br>(Китай, Tonghua, клещ)<br>OR148882, OR148881, OR148883 | 98.29 | 99.20 | 96.06 | 98.75 | 96.28 | 99.32 |
| MDJ014 (China, Heilongjiang, human)<br>(Китай, Heilongjiang, человек)<br>PQ220359, PQ220364, PQ220369 | 98.48 | 99.60 | 98.29 | 99.71 | 98.58 | 99.80 |
| MDJ486 (China, Jilin, human)<br>(Китай, Jilin, человек)<br>PQ220361, PQ220366, PQ220371 | 98.17 | 99.40 | 98.62 | 99.63 | 98.14 | 99.60 |
| BT-2155 (Japan, tick)<br>(Япония, клещ)<br>LC790682, LC790681, LC790680 | 98.42 | 99.40 | 98.57 | 99.62 | 98.24 | 99.62 |
| HH011-2020 (Japan, tick)<br>(Япония, человек)<br>LC621360, LC621359, LC621358 | 93.68 | 100.00 | 97.15 | 99.26 | 97.34 | 99.62 |

*concinna* ticks (25/241; 95% CI 7.1–14.8). Rickettsia DNA was also detected in a tick sample positive for YEZV RNA, indicating the possibility of mixed infection with these two TBDs.

## Conclusion

The study is the first to demonstrate the circulation of the recently discovered nairovirus YEZV in the Amur Region and to determine the complete genome nucleotide sequence of the identified genetic variant of YEZV. The data obtained contribute to the understanding of the genetic diversity and phylogeography of this pathogen in Russia and are of direct practical importance for the epidemiological surveillance system. Up-to-date information on the circulation and genetic diversity of nairoviruses that pose a potential danger to humans and animals contributes to the regulation and adjustment of preventive and anti-epidemic measures, as well as the implementation of adequate region-oriented laboratory diagnostics of TBDs. The detection of YEZV circulation in *I. persulcatus* ticks in the Amur Region confirms the necessity for further monitoring of YEZV spread in natural TBD foci in the Far East, especially in areas bordering China.

The high frequency of TBD pathogens in ixodid ticks dictates the necessity for comprehensive laboratory diagnosis of these diseases in individuals who have sought medical attention for tick bites, taking into account the detection of not only known but also newly identified pathogens in endemic areas. This, in turn, highlights the necessity to develop diagnostic test systems with high sensitivity and specificity for the detection of newly discovered pathogens, as well as the active introduction of these diagnostic tools into practical healthcare.

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**Information about the authors:**

**Mikhail Yu. Kartashov✉ –** Ph.D. (Biol.), Leading Researcher, Department of Molecular Virology Head of the Molecular Diagnostics Sector, State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk Region, Russia. E-mail: mikkartash@yandex.ru; https://orcid.org/0000-0002-7857-6822

**Valentina Yu. Kurushina –** Junior Researcher, Department of Molecular Virology State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk Region, Russia. E-mail: kurushina.valenti@gmail.com; https://orcid.org/0009-0005-8148-5242

**Alina S. Zheleznova –** Junior Researcher, Department of Molecular Virology State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk Region, Russia. E-mail: zheleznova_as@vector.nsc.ru; https://orcid.org/0009-0006-1252-9616

**Kirill A. Svirin –** Junior Researcher, Department of Molecular Virology State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk Region, Russia. E-mail: svirin_ka@vector.nsc.ru; https://orcid.org/0000-0001-9083-1649

**Ekaterina I. Materikina –** Researcher, Department of Molecular Virology State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk Region, Russia. E-mail: katr962@mail.ru; https://orcid.org/0000-0001-5181-0415

**Vladimir A. Ternovoi –** Ph.D. (Biol.), Leading Researcher, Department of Molecular Virology Head of the Laboratory of Molecular Epidemiology, State Research Center of Virology and Biotechnology «Vector», Koltsovo, Novosibirsk Region, Russia. E-mail: tern@vector.nsc.ru; https://orcid.org/0000-0003-1275-171X

**Contribution:** Kartashov M.Yu. – concept and design of the study, conducting research, writing the text of the article; Kurushina V.Yu. Zheleznova A.S., Svirin K.A., Materikina E.I. – performing the experiments; Ternovoi V.A. – research management.

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**Информация об авторах:**

**Карташов Михаил Юрьевич✉ –** канд. биол. наук, ведущий научный сотрудник отдела молекулярной вирусологии, заведующий сектором молекулярной диагностики ФБУН ГНЦ ВБ «Вектор» Роспотребнадзора, р.п. Кольцово, Россия. E-mail: mikkartash@yandex.ru; https://orcid.org/0000-0002-7857-6822

**Курушина Валентина Юрьевна –** стажер-исследователь отдела молекулярной вирусологии ФБУН ГНЦ ВБ «Вектор» Роспотребнадзора, р.п. Кольцово, Россия. E-mail: kurushina.valenti@gmail.com; https://orcid.org/0009-0005-8148-5242

**Железнова Алина Сергеевна –** стажер-исследователь отдела молекулярной вирусологии ФБУН ГНЦ ВБ «Вектор» Роспотребнадзора, р.п. Кольцово, Россия. E-mail: zheleznova_as@vector.nsc.ru; https://orcid.org/0009-0006-1252-9616

**Свирин Кирилл Андреевич –** младший научный сотрудник отдела молекулярной вирусологии ФБУН ГНЦ ВБ «Вектор» Роспотребнадзора, р.п. Кольцово, Россия. E-mail: svirin_ka@vector.nsc.ru; https://orcid.org/0000-0001-9083-1649

**Материкина Екатерина Ильинична –** научный сотрудник отдела молекулярной вирусологии ФБУН ГНЦ ВБ «Вектор» Роспотребнадзора, р.п. Кольцово, Россия. E-mail: katr962@mail.ru; https://orcid.org/0000-0001-5181-0415

**Терновой Владимир Александрович –** канд. биол. наук, ведущий научный сотрудник отдела молекулярной вирусологии, заведующий лабораторией молекулярной эпидемиологии ФБУН ГНЦ ВБ «Вектор» Роспотребнадзора, р.п. Кольцово, Россия. E-mail: tern@vector.nsc.ru; https://orcid.org/0000-0003-1275-171X

**Участие авторов:** Карташов М.Ю. – концепция и дизайн исследования, проведение лабораторных исследований, написание текста статьи; Курушина В.Ю., Железнова А.С., Свирин К.А., Материкина Е.И. – проведение лабораторных исследований; Терновой В.А. – общее руководство, научное редактирование.

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