

Transboundary Movement of Yezo Virus via Ticks on Migratory Birds, Japan, 2020–2021

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Migratory birds carry ticks harboring various pathogens, including the zoonotic Yezo virus. In Hokkaido, Japan, we collected ticks from migratory birds during 2020–2021. Eight of 385 pools, comprising 2,534 ticks, tested positive for Yezo virus RNA, suggesting Yezo virus might be spread through the flyways of migratory birds.

Yezo virus (YEZV), in the order Bunyvirales, family Nairoviridae, genus *Orthonairovirus*, possesses a negative-sense single-stranded RNA genome comprising 3 segments: large (L), medium (M), and small (S) (1). Each segment contains a single open reading frame encoding the RNA-dependent RNA polymerase (L segment), glycoprotein precursor (M segment), and nucleoprotein (S segment) (1). YEZV infection is an emerging infectious disease, detected in Hokkaido, Japan, in 2019 among patients who had febrile illness after a tick bite (1); ≥ 9 patients infected with YEZV have been reported in Hokkaido (1–3). Among those patients, fever, myalgia, thrombocytopenia, leukopenia, and increasing liver enzymes were commonly reported after a tick bite (1–3). One patient who had a YEZV infection after a tick bite has also reported in Inner Mongolia in northeastern China (4).

Migratory birds carry ticks harboring various pathogens, such as Crimean-Congo hemorrhagic

fever virus, belonging to the family *Nairoviridae* (5–7). Phylogenetic analysis of tickborne severe fever with thrombocytopenia syndrome virus indicated that this virus might be carried by ticks found on migratory birds that fly between China, South Korea, and Japan (8,9). Increasing evidence suggests that migratory birds play a critical role in spreading tickborne pathogens. In archipelagos, such as Japan, understanding transmission of pathogens by migratory birds is critical; however, information on tickborne pathogens carried by migratory birds is lacking. We investigated the prevalence of YEZV in ticks found on migratory birds in Japan to determine virus transmission pathways.

The Study

We conducted this research under approval by the animal research review board of Yamashina Institute for Ornithology, Chiba, Japan (approval nos. 2020-004 and 2021-002). We collected ticks infesting birds that mostly fly from Sakhalin and the Kuril Islands to Hokkaido, Japan. We collected the ticks during October 2–12, 2020, and October 2–13, 2021, in Lake Kutcharo, Hamatonbetsu Town, and Lake Furen, Nemuro City, Japan (Figure 1). We analyzed YEZV genes in the ticks to clarify virus spread. Because migratory birds at these locations in autumn are considered to have just arrived from Sakhalin (Sakhalin route) and the Kuril Islands (Kuril Islands route) (Figure 1), it is likely that the ticks crossed the sea attached to the birds.

We collected 2,534 ticks from 15 species of birds in October 2020 and 2021 (Appendix Tables 1, 2; <https://wwwnc.cdc.gov/EID/article/30/12/24-0539-App1.pdf>). All ticks were morphologically identified and pooled according to their species, life stage, and host species. Each pool consisted of ≤ 10 larval or 5 nymphal ticks (Appendix).

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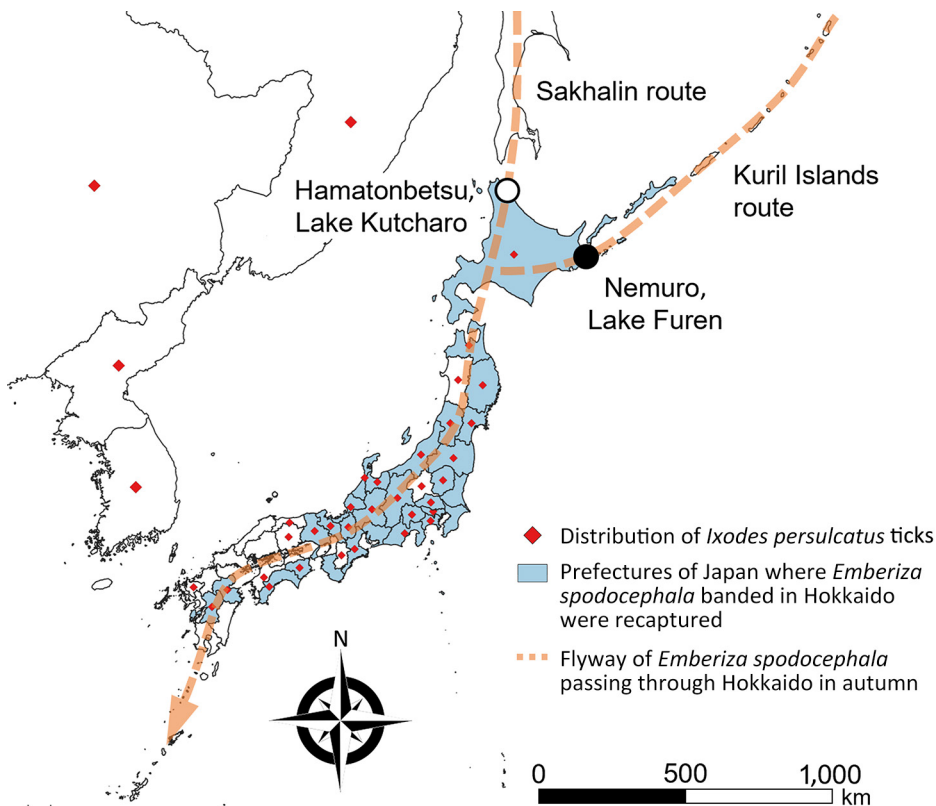


Figure 1. Migratory bird flyways and distribution of *Ixodes persulcatus* ticks in study of transboundary movement of Yezo virus via ticks on birds, Japan, 2020–2021. Distribution of *I. persulcatus* ticks (red rhombus), prefectures of Japan where black-faced buntings (*Emberiza spodocephala*) banded in Hokkaido were recaptured (light blue), and flyway of *E. spodocephala* passing through Hokkaido during autumn (orange dotted line). Distribution outside Japan is indicated at the country level. Black circle shows Lake Furen, Nemuro City, Hokkaido; white circle shows Lake Kutcharo, Hamatonbetsu Town, Hokkaido.

We examined 2,323 *Ixodes persulcatus* ticks (1,727 larvae and 596 nymphs), 203 *I. pavloovskyi* ticks (169 larvae and 34 nymphs), 1 *I. turdus* tick (nymph), 3 *Haemaphysalis megaspinoso* ticks (larvae), and 4 *H. concinna* ticks (3 larvae and 1 nymph) by using quantitative reverse transcription PCR (Appendix). Of those, 8 pools of *I. persulcatus* ticks were positive for YEZV RNA; no pools for the other tick species were YEZV positive (overall minimum infection rate 0.3%) (Table 1). Seven of 8 positive pools showed low cycle threshold (Ct) values of 22.08–30.08; 1 pool had a high Ct value of 39.19. Seven pools with low Ct values were used for further analysis. Ticks in all positive pools were *I. persulcatus* collected from black-faced buntings (*Emberiza spodocephala*) (Table 2). Five pools were collected in Lake Furen, Nemuro City, Hokkaido, and the other pools were collected in Lake Kutcharo, Hamatonbetsu Town, Hokkaido. Results indicated that ticks with YEZV were transferred by migratory birds along both the Sakhalin and Kuril Islands routes.

In *I. persulcatus* ticks, the minimum infection rate of YEZV was 0.2% in larvae and 0.8% in nymphs (Table 1). YEZV has been reported in 3 adult tick species, *H. megaspinoso*, *I. ovatus*, and *I. persulcatus*, found on vegetation in Hokkaido (1). In addition, 4 YEZV-positive pools consisted of only unfed ticks

(Appendix Table 3), indicating that *I. persulcatus* might transmit Yezo virus.

We sequenced 7 complete genomes of YEZV: YEZV/tick/BT-1821/Japan/2020, YEZV/tick/BT-1826/Japan/2020, YEZV/tick/BT-1844/Japan/2020, YEZV/tick/BT-1864/Japan/2020, YEZV/tick/BT-1968/Japan/2020, YEZV/tick/BT-2135/Japan/2021, and YEZV/tick/BT-2155/Japan/2021 (Appendix) and deposited those sequences into the DNA Data Bank of Japan (<https://www.ddbj.nig.ac.jp>) (Table 2). For 1 pool that had a high Ct value, YEZV/tick/BT-1822/Japan/2020, we could not determine the complete genome sequence; however, we amplified a short fragment of the S segment by nested reverse transcription PCR and deposited that sequence in the DNA Data Bank of Japan (Table 2).

We analyzed the nucleotide and amino acid sequences of the RNA-dependent RNA polymerase, glycoprotein precursor, and nucleoprotein among YEZV strains and compared those sequences to Sulina virus IxriSL16-01 sequences (10) (Appendix Tables 4–6). Sequence identity between YEZV strains was 93.0%–100.0% at the nucleotide level and 99.2%–100.0% at the amino acid level.

We also performed phylogenetic analysis of YEZV strains by using the nucleotide sequences of the

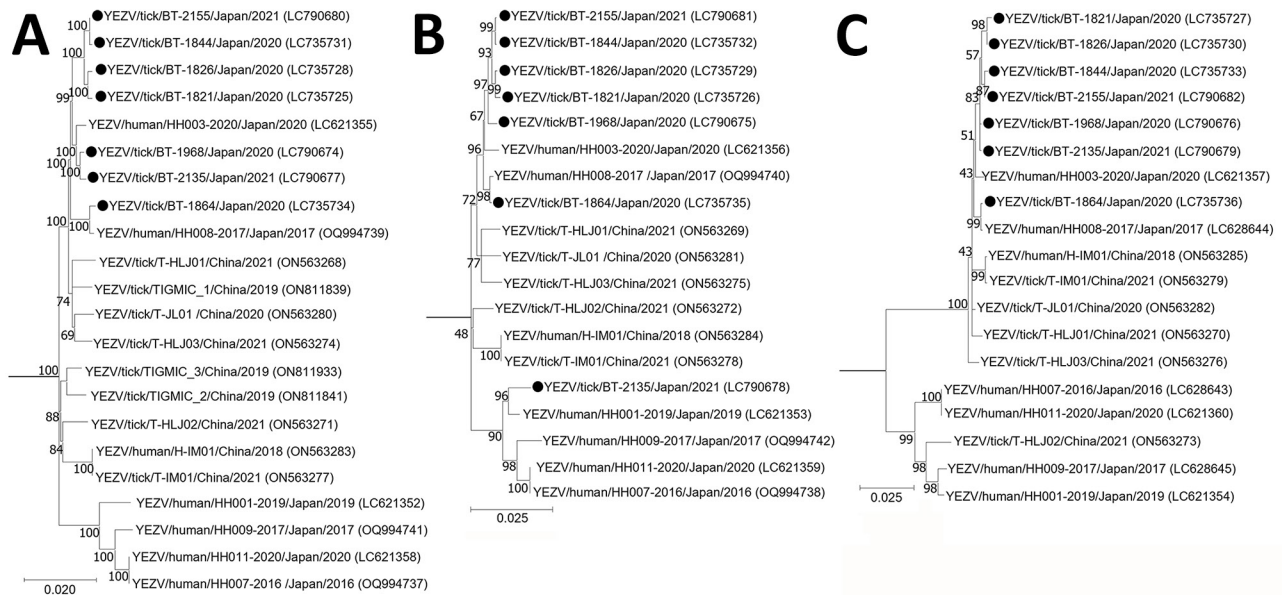


Figure 2. Phylogenetic analysis of Yezo virus strains in study of transboundary movement of Yezo virus via ticks on migratory birds, Japan, 2020–2021. Trees were constructed by using the maximum-likelihood method in MEGA X (<https://www.megasoftware.net>) and 1,000 bootstrap replicates for nucleotide sequences. A) Large segment; B) medium segment; C) small segment. Black circles indicate sequences from this study. The number at each branch indicates the bootstrap value. GenBank accession numbers for nucleotide sequences are shown in parentheses. *Sulina virus* IxriSL16-01 was used as the outgroup to determine the root of Yezo virus trees but is not shown. Scale bars indicate nucleotide substitutions per site.

open reading frames of the L, M, and S segments (Figure 2). All tick-derived strains detected in this study belonged to the same cluster as the strains obtained from patients in Hokkaido, YEZV/human/HH003-2020/Japan/2020 and YEZV/human/HH008-2017/Japan/2017 (1). In addition, our tick-derived strain, YEZV/tick/BT-2135/Japan/2021, and the tick-derived strain from Heilongjiang, China, YEZV/tick/T-HLJ02/People's Republic of China/2021, were reassortment strains; both were generated through reassortment with the strains found in patients in Hokkaido, Japan. We also confirmed the events of

genetic reassortment by using a recombination detection program (A. Nishino and K. Maeda, unpub. data), which suggested that YEZV might be transferred between China and Hokkaido, Japan.

Conclusions

We successfully detected YEZV in *I. persulcatus* ticks from migratory birds (*E. spodocephala* buntings) flying to Hokkaido, Japan, from Sakhalin and the Kuril Islands (5,11). The distribution of *I. persulcatus* ticks partially overlapped with the flyway of *E. spodocephala* buntings passing through Hokkaido in autumn (12–15). Together

Table 1. Virus prevalence in tick species collected in an investigation of transboundary movement of Yezo virus via ticks on migratory birds, Japan, 2020–2021

Species	No. ticks examined	No. pools examined	No. positive pools	Minimum infection rate, %
<i>Ixodes persulcatus</i>				
Larva	1,727	193	3	0.2
Nymph	596	137	5	0.8
<i>I. pavlovskyi</i>				
Larva	169	30	0	0
Nymph	34	19	0	0
<i>I. turdus</i>				
Larva	0	0	0	0
Nymph	1	1	0	0
<i>Haemaphysalis megaspinosa</i>				
Larva	3	2	0	0
Nymph	0	0	0	0
<i>H. concinna</i>				
Larva	3	2	0	0
Nymph	1	1	0	0
Total	2,534	385	8	0.3

Table 2. Genomic sequences in an investigation of transboundary movement of Yezo virus via ticks on migratory birds, Japan, 2020–2021*

Virus strain	Tick stage	No. ticks in pool	Collection site	Collection date	Segment	Accession nos.†
YEZV/tick/BT-1821/Japan/2020	Nymph	5	Lake Kutcharo, Hamatonbetsu	2020 Oct 6–7	L	LC735725
					M	LC735726
					S	LC735727
YEZV/tick/BT-1822/Japan/2020	Nymph	5	Lake Kutcharo, Hamatonbetsu	2020 Oct 6–7	S	LC737964, partial sequence
YEZV/tick/BT-1826/Japan/2020	Nymph	5	Lake Kutcharo, Hamatonbetsu	2020 Oct 7	L	LC735728
					M	LC735729
					S	LC735730
YEZV/tick/BT-1844/Japan/2020	Larva	10	Lake Furen, Nemuro	2020 Oct 8	L	LC735731
					M	LC735732
					S	LC735733
YEZV/tick/BT-1864/Japan/2020	Larva	10	Lake Furen, Nemuro	2020 Oct 8	L	LC735734
					M	LC735735
					S	LC735736
YEZV/tick/BT-1968/Japan/2020	Larva	10	Lake Furen, Nemuro	2020 Oct 11	L	LC790674
					M	LC790675
					S	LC790676
YEZV/tick/BT-2135/Japan/2021	Nymph	5	Lake Furen, Nemuro	2021 Oct 13	L	LC790677
					M	LC790678
					S	LC790679
YEZV/tick/BT-2155/Japan/2021	Nymph	5	Lake Furen, Nemuro	2021 Oct 13	L	LC790680
					M	LC790681
					S	LC790682

*All ticks collected were *Ixodes persulcatus* and all birds collected were black-faced buntings (*Emberiza spodocephala*). L, large segment; M, medium segment; S, small segment.

†DNA Data Bank of Japan (<https://www.ddbj.nig.ac.jp>) accession numbers.

with the possible genetic reassortment event between YEZV strains from Japan and China, this finding indicated that YEZV might be carried by migratory birds from other countries to Japan and from Hokkaido to other prefectures in Japan. To elucidate the distribution area and transmission routes of YEZV, further surveillance of YEZV infection should be conducted in ticks, birds, and wild and domestic animals.

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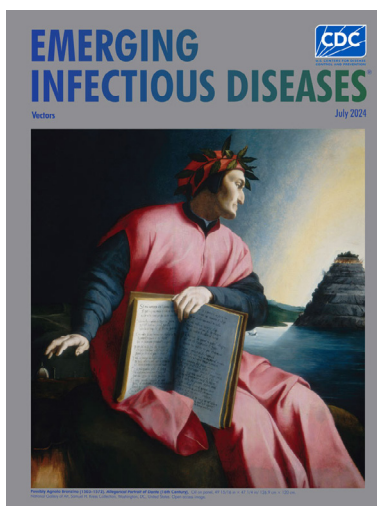
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Appendix

Additional Methods

Homogenization of Ticks

The pooled ticks were homogenized in 500 μ L of phosphate-buffered saline by using a BioMasher II (Nippi. Inc., <https://www.nippi-inc.co.jp>). The homogenates were centrifuged at $6,300 \times g$ at 4°C for 10 minutes, and supernatants were filtrated and stored at –80°C until use.

RNA Extraction and Quantitative Reverse Transcription PCR (qRT-PCR)

RNA was extracted from each supernatant by using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific, <https://www.thermofisher.com>). Yezo virus S segment RNAs were amplified by using the QuantiTect Probe RT-PCR Kit (primer 1: 5'-AGCCCTTGACACTGCATTT-3', primer 2: 5'-CATACAGGAAGGCCATCTCATT-3', and probe: 6-FAM-ACCTACTACTGGATGTGGAAGGCAGA-3IABkFQ) (QIAGEN, <https://www.qiagen.com>) under the following thermal conditions: 50°C for 30 minutes, 95°C for 15 minutes, and 45 cycles of 94°C for 15 seconds and 60°C for 60 seconds.

Determination of Yezo Virus Complete Genome Sequences

To determine the complete genome sequences of Yezo viruses (YEZVs), we performed qRT-PCR by using a series of primer sets designed according to deposited nucleotide sequences of YEZV strains. RT-PCR was performed by first using the One Step RT-PCR Kit (QIAGEN), or cDNA was synthesized from RNA by using SuperScript III First-Strand Synthesis SuperMix (Thermo Fisher Scientific); PCR was performed by using Tks Gflex DNA Polymerase (Takara Bio, <https://www.takarabio.com>). Sanger sequencing was performed by Eurofins Genomics, Japan, (<https://www.eurofins.com>) on purified PCR products from 4 viruses. The other 3 viruses were sequenced by using next-generation sequencing, and libraries of the purified PCR products were constructed by using the QIAseq FX DNA Library Kit (QIAGEN). The libraries were pooled and purified by using Agencourt AMPure XP (Beckman Coulter, Inc., <https://www.beckman.com>). Sequencing was performed by using MiSeq and MiSeq Reagent Nano Kit v2 (both Illumina, <https://www.illumina.com>). All reads were mapped to the YEZV HH003-2020 strain and consensus sequences were obtained by using CLC Genomics Workbench software (QIAGEN).

Appendix Table 1. Information on migratory birds captured in Japan, 2020, and the infesting ticks

Species of migratory bird	No. captured birds			No. birds infested by ticks*			No. ticks*		
	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total
<i>Emberiza spodocephala</i>	693	1,142	1,835	129	519	648	185	991	1,176
<i>Horornis diphone</i>	100	36	136	15	2	17	16	2	18
<i>Turdus chrysolais</i>	4	35	39	2	17	19	2	26	28
<i>Tarsiger cyanurus</i>	0	17	17	0	5	5	0	5	5
<i>Emberiza variabilis</i>	4	10	14	1	4	5	4	9	13
<i>Luscinia calliope</i>	6	2	8	3	1	4	6	2	8
<i>Sitta europaea</i>	3	0	3	1	0	1	1	0	1
<i>Troglodytes troglodytes</i>	0	3	3	0	2	2	0	4	4
<i>Erithacus akahige</i>	0	1	1	0	1	1	0	3	3
<i>Phylloscopus schwarzi</i>	1	0	1	1	0	1	2	0	2
<i>Turdus obscurus</i>	1	0	1	1	0	1	1	0	1
Total	812	1,246	2,058	153	551	704	217	1,042	1,259

*Only live ticks on migratory birds were counted.

Appendix Table 2. Information on migratory birds captured in Japan, 2021, and the infesting ticks

Species of migratory bird	No. captured birds			No. birds infested by ticks*			No. ticks*		
	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total
<i>Emberiza spodocephala</i>	651	1,098	1,749	157	421	578	273	896	1,169
<i>Uragus sibiricus</i>	0	61	61	0	1	1	0	1	1
<i>Horornis diphone</i>	51	8	59	9	1	10	9	1	10
<i>Turdus chrysolais</i>	8	24	32	4	20	24	7	37	44
<i>Emberiza variabilis</i>	7	9	16	4	4	8	13	17	30
<i>Tarsiger cyanurus</i>	0	8	8	0	4	4	0	6	6
<i>Certhia familiaris</i>	2	4	6	1	1	2	1	1	2
<i>Parus minor</i>	6	0	6	1	0	1	1	0	1
<i>Troglodytes troglodytes</i>	0	6	6	0	4	4	0	7	7
<i>Parus palustris</i>	0	5	5	0	1	1	0	1	1
<i>Luscinia calliope</i>	4	0	4	1	0	1	2	0	2
<i>Sitta europaea</i>	0	2	2	0	1	1	0	1	1
<i>Turdus obscurus</i>	1	0	1	1	0	1	1	0	1
Total	730	1,225	1,955	178	458	636	307	968	1,275

*Only live ticks on migratory birds were counted.

Appendix Table 3. Information on ticks from which Yezo virus was detected in a study of ticks collected from migratory birds, Japan, 2020–2021

Strain	Tick species	Stage	No. ticks in pool	Status of bloodfeeding		
				Engorged	Unfed	Engorged or unfed
YEZV/tick/BT-1821/Japan/2020	<i>Ixodes persulcatus</i>	Nymph	5	1	4	0
YEZV/tick/BT-1822/Japan/2020	<i>Ixodes persulcatus</i>	Nymph	5	1	4	0
YEZV/tick/BT-1826/Japan/2020	<i>Ixodes persulcatus</i>	Nymph	5	0	5	0
YEZV/tick/BT-1844/Japan/2020	<i>Ixodes persulcatus</i>	Larva	10	3	6	1
YEZV/tick/BT-1864/Japan/2020	<i>Ixodes persulcatus</i>	Larva	10	0	10	0
YEZV/tick/BT-1968/Japan/2020	<i>Ixodes persulcatus</i>	Larva	10	3	1	6
YEZV/tick/BT-2135/Japan/2021	<i>Ixodes persulcatus</i>	Nymph	5	0	5	0
YEZV/tick/BT-2155/Japan/2021	<i>Ixodes persulcatus</i>	Nymph	5	0	5	0

Appendix Table 4. Pairwise comparison of the nucleotide and amino acid sequences of the RNA-dependent RNA polymerase of Yezo virus strains and Sulina virus IxriSL16-01*

Virus	YEZV/tick /BT- 1821/Jap an/2020	YEZV/tick /BT- 1826/Jap an/2020	YEZV/tick/ BT- 1844/Jap an/2020	YEZV/tick /BT- 1864/Jap an/2020	YEZV/tick /BT- 1968/Jap an/2020	YEZV/tick/ BT- 2135/Jap an/2021	YEZV/tick /BT- 2155/Jap an/2021	YEZV/hu man/HH0 01- 2019/Jap an/2019	YEZV/hu man/HH0 03- 2020/Jap an/2020	YEZV/hu man/HH0 07-2016 /Japan/20 16	YEZV/hu man/HH0 08- 2017/Jap an/2017	YEZV/hu man/HH0 09- 2017/Jap an/2017	YEZV/hu man/HH0 11- 2020/Jap an/2020	YEZV/hu man/H- IM01/Chin a/2018	YEZV/tick/ T- HLJ01/Ch ina/2021	YEZV/tick /T- HLJ02/Ch ina/2021	YEZV/tick /T- HLJ03/Ch ina/2021	YEZV/tick/ TIGMIC_1 /China/20 19	YEZV/tick /TIGMIC_2 /China/2 019	YEZV/tick /TIGMIC_3 /China/2 019	YEZV/tick/ T- IM01/Chin a/2021	YEZV/tick /T-JL01 /China/20 20	Sulina virus IxriSL16- 01	
YEZV/tick/BT-1821/Japan/2020	—	99.8	99.6	98.8	99.1	99.1	99.6	97.3	99.1	97.4	98.8	97.2	97.4	98.4	98.7	98.4	98.8	98.7	98.4	98.5	98.4	98.8	72.8	
YEZV/tick/BT-1826/Japan/2020	99.9	—	99.6	98.8	99.1	99.1	99.6	97.2	99.1	97.4	98.8	97.2	97.4	98.3	98.6	98.4	98.8	98.7	98.4	98.5	98.3	98.7	72.8	
YEZV/tick/BT-1844/Japan/2020	99.9	99.9	—	98.8	99.1	99.1	99.9	97.2	99.1	97.4	98.8	97.2	97.4	98.3	98.6	98.4	98.8	98.7	98.4	98.5	98.3	98.7	72.8	
YEZV/tick/BT-1864/Japan/2020	99.4	99.4	99.5	—	98.9	98.9	98.8	97.3	98.9	97.3	99.7	97.2	97.3	98.1	98.6	98.3	98.7	98.6	98.3	98.4	98.1	98.7	72.8	
YEZV/tick/BT-1968/Japan/2020	99.8	99.8	99.9	99.6	—	99.7	99.2	97.5	99.5	97.6	99.0	97.4	97.6	98.4	98.8	98.5	98.9	98.9	98.6	98.6	98.4	98.9	72.8	
YEZV/tick/BT-2135/Japan/2021	99.7	99.7	99.8	99.6	99.9	—	99.1	97.4	99.4	97.5	98.9	97.4	97.5	98.4	98.7	98.5	98.9	98.8	98.5	98.6	98.3	98.9	72.8	
YEZV/tick/BT-2155/Japan/2021	99.9	99.9	100.0	99.5	99.8	99.8	—	97.3	99.1	97.4	98.8	97.2	97.4	98.4	98.6	98.4	98.8	98.8	98.5	98.5	98.3	98.8	72.8	
YEZV/human/HH001-2019/Japan/2019	99.5	99.5	99.6	99.5	99.8	99.7	99.6	—	97.4	98.4	97.2	98.3	98.4	97.4	97.2	97.4	97.4	97.2	97.4	97.5	97.3	97.3	72.9	
YEZV/human/HH003-2020/Japan/2020	99.7	99.7	99.8	99.6	99.9	99.9	99.8	99.7	—	97.5	98.9	97.4	97.5	98.4	98.7	98.5	98.9	98.8	98.5	98.6	98.3	98.9	72.8	
YEZV/human/HH007-2016/Japan/2016	99.5	99.5	99.6	99.4	99.7	99.7	99.6	99.8	99.6	—	97.3	99.2	100.0	97.5	97.3	97.5	97.5	97.4	97.6	97.6	97.4	97.4	72.9	
YEZV/human/HH008-2017/Japan/2017	99.5	99.5	99.6	99.9	99.8	99.7	99.6	99.6	99.7	99.5	—	97.2	97.3	98.2	98.6	98.3	98.7	98.7	98.3	98.4	98.1	98.7	72.8	
YEZV/human/HH009-2017/Japan/2017	99.4	99.4	99.5	99.3	99.6	99.5	99.4	99.7	99.5	99.7	99.4	—	99.2	97.3	97.2	97.4	97.4	97.2	97.4	97.4	97.3	97.3	72.8	
YEZV/human/HH011-2020/Japan/2020	99.5	99.5	99.6	99.4	99.7	99.7	99.6	99.8	99.6	100.0	99.5	99.7	—	97.5	97.3	97.5	97.5	97.4	97.6	97.6	97.4	97.4	72.9	
YEZV/human/H-IM01/China/2018	99.5	99.5	99.6	99.5	99.7	99.7	99.6	99.7	99.6	99.6	99.6	99.5	99.6	—	98.2	98.5	98.4	98.3	98.5	98.6	100.0	98.3	72.4	
YEZV/tick/T-HLJ01/China/2021	99.6	99.6	99.8	99.5	99.9	99.8	99.7	99.7	99.8	99.6	99.6	99.5	99.6	99.7	—	98.5	98.9	98.8	98.4	98.4	98.2	98.8	72.6	
YEZV/tick/T-HLJ02/China/2021	99.6	99.6	99.7	99.5	99.8	99.8	99.7	99.8	99.7	99.7	99.6	99.6	99.7	99.7	99.8	—	98.4	98.4	98.6	98.7	98.5	98.3	72.5	
YEZV/tick/T-HLJ03/China/2021	99.6	99.6	99.7	99.5	99.8	99.8	99.6	99.6	99.7	99.6	99.6	99.4	99.6	99.6	99.8	99.7	—	99.0	98.5	98.5	98.3	99.0	72.6	
YEZV/tick/TIGMIC_1/China/2019	99.7	99.7	99.8	99.5	99.8	99.8	99.8	99.7	99.8	99.7	99.6	99.5	99.7	99.7	99.8	99.8	99.8	—	98.4	98.5	98.2	98.9	72.8	
YEZV/tick/TIGMIC_2/China/2019	99.6	99.6	99.7	99.5	99.8	99.8	99.7	99.8	99.8	99.7	99.6	99.6	99.7	99.8	99.8	99.8	99.7	99.8	—	99.0	98.5	98.4	72.9	
YEZV/tick/TIGMIC_3/China/2019	99.7	99.7	99.8	99.6	99.9	99.9	99.8	99.8	99.8	99.8	99.7	99.6	99.8	99.8	99.8	99.9	99.8	99.9	100.0	—	98.5	98.5	72.9	
YEZV/tick/T-IM01/China/2021	99.5	99.5	99.6	99.5	99.7	99.7	99.6	99.7	99.6	99.6	99.6	99.5	99.6	100.0	99.7	99.7	99.6	99.7	99.8	99.8	—	98.3	72.4	
YEZV/tick/T-JL01/China/2020	99.5	99.5	99.6	99.4	99.8	99.7	99.6	99.6	99.7	99.5	99.5	99.4	99.5	99.6	99.8	99.6	99.8	99.7	99.7	99.7	99.6	—	72.8	
Sulina virus IxriSL16-01	82.1	82.2	82.2	82.0	82.1	82.1	82.1	82.2	82.1	82.2	82.1	82.1	82.2	82.0	82.2	82.1	82.1	82.1	82.1	82.1	82.1	82.0	82.1	—

*Nucleotide sequence identity (%) is shown above the diagonal and amino acid sequence identity (%) is shown below the diagonal.

Appendix Table 5. Pairwise comparison of the nucleotide and amino acid sequences of the glycoprotein precursor of Yezo virus strains and Sulina virus IxriSL16-01*

Virus	YEZV/huma																			Sulina virus IxriSL16-01
	YEZV/tick/B T- 1821/Japan /2020	YEZV/tick/B T- 1826/Japan /2020	YEZV/tick/B T- 1844/Japan /2020	YEZV/tick/B T- 1864/Japan /2020	YEZV/tick/B T- 1968/Japan /2020	YEZV/tick/B T- 2135/Japan /2021	YEZV/tick/B T- 2155/Japan /2021	YEZV/huma n/HH001- 2019/Japan /2019	YEZV/huma n/HH003- 2020/Japan /2020	YEZV/huma n/HH007- 2016 /Japan/2016	YEZV/huma n/HH008- 2017/Japan /2017	YEZV/huma n/HH009- 2017/Japan /2017	YEZV/huma n/HH011- 2020/Japan /2020	YEZV/huma n/H- IM01/China/ 2018	YEZV/tick/T - HLJ01/Chin a/2021	YEZV/tick/T - HLJ02/Chin a/2021	YEZV/tick/T - HLJ03/Chin a/2021	YEZV/tick/T - IM01/China/ 2021	YEZV/tick/T - JL01 /China/2020	
YEZV/tick/BT- 1821/Japan/2020	—	99.8	99.6	99.3	99.3	97.5	99.6	97.8	99.1	97.5	99.2	97.1	97.5	98.3	98.6	98.5	98.6	98.3	98.5	68.7
YEZV/tick/BT- 1826/Japan/2020	99.8	—	99.7	99.4	99.4	97.5	99.7	97.8	99.2	97.5	99.3	97.1	97.5	98.3	98.7	98.6	98.7	98.3	98.6	68.8
YEZV/tick/BT- 1844/Japan/2020	99.6	99.8	—	99.4	99.5	97.5	100.0	97.8	99.2	97.5	99.3	97.2	97.5	98.3	98.7	98.6	98.7	98.3	98.7	68.9
YEZV/tick/BT- 1864/Japan/2020	99.6	99.9	99.6	—	99.3	97.6	99.4	98.0	99.3	97.6	99.8	97.3	97.6	98.5	98.9	98.7	98.8	98.5	98.8	69.1
YEZV/tick/BT- 1968/Japan/2020	99.5	99.7	99.6	99.6	—	97.5	99.5	97.9	99.2	97.5	99.3	97.2	97.5	98.3	98.7	98.5	98.6	98.3	98.6	69.6
YEZV/tick/BT- 2135/Japan/2021	99.2	99.4	99.3	99.3	99.2	—	97.5	99.0	97.5	98.3	97.6	98.0	98.3	97.5	97.5	97.7	97.4	97.5	97.4	69.0
YEZV/tick/BT- 2155/Japan/2021	99.6	99.9	99.9	99.7	99.7	99.3	—	97.8	99.2	97.5	99.3	97.2	97.5	98.3	98.7	98.6	98.7	98.3	98.7	68.9
YEZV/human/HH001- 2019/Japan/2019	99.4	99.6	99.5	99.5	99.4	99.5	99.6	—	97.9	98.7	97.9	98.3	98.7	97.8	97.8	98.0	97.7	97.8	97.7	69.7
YEZV/human/HH003- 2020/Japan/2020	99.6	99.7	99.6	99.6	99.5	99.3	99.6	99.5	—	97.4	99.2	97.1	97.4	98.2	98.7	98.5	98.6	98.2	98.6	69.1
YEZV/human/HH007- 2016 /Japan/2016	99.4	99.6	99.5	99.5	99.4	99.5	99.6	99.7	99.5	—	97.5	98.8	100.0	97.4	97.4	97.6	97.3	97.4	97.4	69.8
YEZV/human/HH008- 2017/Japan/2017	99.5	99.7	99.6	99.9	99.5	99.3	99.6	99.5	99.6	99.5	—	97.2	97.5	98.5	98.9	98.6	98.7	98.5	98.7	69.1
YEZV/human/HH009- 2017/Japan/2017	99.3	99.6	99.4	99.4	99.3	99.4	99.5	99.6	99.4	99.6	99.4	—	98.8	97.2	97.2	97.4	97.0	97.2	97.1	69.7
YEZV/human/HH011- 2020/Japan/2020	99.4	99.6	99.5	99.5	99.4	99.5	99.6	99.7	99.5	100.0	99.5	99.6	—	97.4	97.4	97.6	97.3	97.4	97.4	69.8
YEZV/human/H- IM01/China/2018	99.4	99.6	99.5	99.5	99.4	99.3	99.6	99.6	99.5	99.6	99.5	99.5	99.6	—	98.3	98.6	98.2	100.0	98.2	69.2
YEZV/tick/T- HLJ01/China/2021	99.5	99.7	99.6	99.6	99.5	99.3	99.6	99.5	99.6	99.5	99.6	99.4	99.5	99.5	—	98.5	98.8	98.3	98.9	68.5
YEZV/tick/T- HLJ02/China/2021	99.6	99.8	99.6	99.6	99.6	99.5	99.7	99.7	99.6	99.7	99.6	99.6	99.7	99.7	99.6	—	98.3	98.6	98.4	69.1
YEZV/tick/T- HLJ03/China/2021	99.6	99.8	99.6	99.6	99.6	99.3	99.7	99.6	99.6	99.6	99.6	99.5	99.6	99.6	99.6	99.7	—	98.2	98.8	69.3
YEZV/tick/T- IM01/China/2021	99.4	99.6	99.5	99.5	99.4	99.3	99.6	99.6	99.5	99.6	99.5	99.5	99.6	100.0	99.5	99.7	99.6	—	98.2	69.2
YEZV/tick/T-JL01 /China/2020	99.5	99.7	99.6	99.6	99.5	99.3	99.6	99.5	99.6	99.5	99.6	99.4	99.5	99.6	99.6	99.6	99.6	99.6	—	69.1
Sulina virus IxriSL16- 01	57.0	57.1	57.1	57.0	57.2	57.0	57.1	57.1	57.1	57.1	57.1	57.0	57.3	57.1	57.1	57.1	57.0	57.3	57.1	—

*Nucleotide sequence identity (%) is shown above the diagonal and amino acid sequence identity (%) is shown below the diagonal.

Appendix Table 6. Pairwise comparison of the nucleotide and amino acid sequences of the nucleoprotein of Yezo virus strains and Sulina virus IxriSL16-01*

Virus	YEZV/hu																			Sulina virus IxriSL16-01
	YEZV/tick/ BT- 1821/Japa n/2020	YEZV/tick/ BT- 1826/Japa n/2020	YEZV/tick/ BT- 1844/Japa n/2020	YEZV/tick/ BT- 1864/Japa n/2020	YEZV/tick/ BT- 1968/Japa n/2020	YEZV/tick/ BT- 2135/Japa n/2021	YEZV/tick/ BT- 2155/Japa n/2021	YEZV/hu man/HH001- 2019/Japa n/2019	YEZV/hu man/HH003- 2020/Japa n/2020	YEZV/hu man/HH007- 2016 /Japan/2016	YEZV/hu man/HH008- 2017/Japan /2017	YEZV/hu man/HH009- 2017/Japa n/2017	YEZV/hu man/HH011- 2020/Japa n/2020	YEZV/huma n/H- IM01/China/ 2018	YEZV/tick /T- HLJ01/Ch ina/2021	YEZV/tick/ T- HLJ02/Chi na/2021	YEZV/tick/ T- HLJ03/Chi na/2021	YEZV/tick/ T- IM01/China /2021	YEZV/tick/ T-JL01 /China/2020	
YEZV/tick/BT- 1821/Japan/2020	—	99.8	99.3	98.8	99.4	99.4	99.4	93.5	98.9	93.4	98.8	93.1	93.4	98.6	98.6	93.1	98.4	98.6	98.9	75.6
YEZV/tick/BT- 1826/Japan/2020	99.8	—	99.5	99.0	99.6	99.6	99.6	93.6	99.1	93.5	99.0	93.2	93.5	98.8	98.8	93.2	98.6	98.8	99.1	75.9
YEZV/tick/BT- 1844/Japan/2020	99.8	100.0	—	99.0	99.6	99.6	99.9	93.7	99.2	93.7	99.0	93.3	93.7	98.8	98.8	93.3	98.6	98.8	99.1	66.6
YEZV/tick/BT- 1864/Japan/2020	99.8	100.0	100.0	—	99.3	99.3	99.1	93.6	99.1	93.4	99.9	93.2	93.4	98.9	99.0	93.2	98.8	98.9	99.3	66.6

Virus	YEZV/tick/ BT- 1821/Japan/ 2020	YEZV/tick/ BT- 1826/Japan/ 2020	YEZV/tick/ BT- 1844/Japan/ 2020	YEZV/tick/ BT- 1864/Japan/ 2020	YEZV/tick/ BT- 1968/Japan/ 2020	YEZV/tick/ BT- 2135/Japan/ 2021	YEZV/tick/ BT- 2155/Japan/ 2021	YEZV/human/ HH001- 2019/Japan/ 2019	YEZV/human/ HH003- 2020/Japan/ 2020	YEZV/human/ HH007- 2016 /Japan/2016	YEZV/human/ HH008- 2017/Japan/ 2017	YEZV/human/ HH009- 2017/Japan/ 2017	YEZV/human/ HH011- 2020/Japan/ 2020	YEZV/human/ H- IM01/China/ 2018	YEZV/tick/ /T- HLJ01/China/ 2021	YEZV/tick/ T- HLJ02/China/ 2021	YEZV/tick/ T- HLJ03/China/ 2021	YEZV/tick/ T- IM01/China/ 2021	YEZV/tick/ T-JL01 /China/2020	Sulina virus IxriSL16-01
YEZV/tick/BT- 1968/Japan/2020	99.8	100.0	100.0	100.0	—	99.9	99.7	93.7	99.3	93.6	99.3	93.3	93.6	99.1	99.1	93.3	98.9	99.1	99.3	66.5
YEZV/tick/BT- 2135/Japan/2021	99.8	100.0	100.0	100.0	100.0	—	99.7	93.7	99.3	93.6	99.3	93.3	93.6	99.1	99.1	93.3	98.9	99.1	99.3	66.4
YEZV/tick/BT- 2155/Japan/2021	99.8	100.0	100.0	100.0	100.0	100.0	—	93.7	99.3	93.8	99.1	93.3	93.8	98.9	98.9	93.3	98.7	98.9	99.2	66.6
YEZV/human/ /HH001- 2019/Japan/2019	99.6	99.8	99.8	99.8	99.8	99.8	99.8	—	93.7	97.5	93.6	99.2	97.6	94.0	93.6	98.1	93.8	93.8	93.7	76.1
YEZV/human/ /HH003- 2020/Japan/2020	99.8	100.0	100.0	100.0	100.0	100.0	100.0	99.8	—	93.6	99.1	93.3	93.6	98.8	98.9	93.4	98.7	98.8	99.2	66.6
YEZV/human/ /HH007-2016 /Japan/2016	99.4	99.6	99.6	99.6	99.6	99.6	99.6	99.8	99.6	—	93.9	97.2	99.9	94.0	93.6	97.2	93.5	93.9	93.7	76.6
YEZV/human/ /HH008- 2017/Japan/2017	99.6	99.8	99.8	99.8	99.8	99.8	99.8	99.6	99.8	99.4	—	93.2	93.9	98.9	99.0	93.2	98.8	98.9	99.3	66.6
YEZV/human/ /HH009- 2017/Japan/2017	99.4	99.6	99.6	99.6	99.6	99.6	99.6	99.8	99.6	99.6	99.4	—	97.3	93.6	93.2	98.0	93.4	93.4	93.3	76.1
YEZV/human/ /HH011- 2020/Japan/2020	99.6	99.8	99.8	99.8	99.8	99.8	99.8	100.0	99.8	99.8	99.6	99.8	—	94.0	93.6	97.2	93.5	93.9	93.7	76.6
YEZV/human/ /H- IM01/China/2018	99.6	99.8	99.8	99.8	99.8	99.8	99.8	99.6	99.8	99.4	99.6	99.4	99.6	—	98.8	93.6	98.6	99.9	99.1	66.4
YEZV/tick/T- HLJ01/China/ 2021	99.6	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.6	99.6	99.6	99.8	99.6	—	93.0	98.9	98.8	99.3	75.6
YEZV/tick/T- HLJ02/China/ 2021	99.6	99.8	99.8	99.8	99.8	99.8	99.8	100.0	99.8	99.8	99.6	99.8	100.0	99.6	99.8	—	93.5	93.4	93.3	75.7
YEZV/tick/T- HLJ03/China/ 2021	99.4	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.4	99.4	99.4	99.6	99.4	99.8	99.6	—	98.6	99.1	66.2
YEZV/tick/T- IM01/China/2021	99.6	99.8	99.8	99.8	99.8	99.8	99.8	99.6	99.8	99.4	99.6	99.4	99.6	100.0	99.6	99.6	99.4	—	99.1	66.5
YEZV/tick/T- JL01 /China/2020	99.6	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.6	99.6	99.6	99.8	99.6	100.0	99.8	99.8	99.6	—	66.4
Sulina virus IxriSL16-01	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.3	60.5	60.3	60.5	60.5	60.5	60.5	60.7	60.5	60.5	—

*Nucleotide sequence identity (%) is shown above the diagonal and amino acid sequence identity (%) is shown below the diagonal.