DOI: https://10.3201/eid3012.240539

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

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

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 × 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 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

	No. capt	ured birds		No. birds infe	sted by tio	cks*	No. ticks*						
Species of	Llomotonhotor	Nemuro		Llomotonhotori	Nemuro		Hamatonbetsu	Nemuro					
migratory	Hamatonbetsu	(Lake	T . 4 . 1	Hamatonbetsu	(Lake	T . 4 . 1	(Lake	(Lake	T . 4 . 1				
bird	(Lake Kutcharo)	Furen)	Total	(Lake Kutcharo)	Furen)	Total	Kutcharo)	Furen)	Total				
Emberiza spodocephala	693	1,142	1,835	129	519	648	185	991	1,176				
Horornis diphone	100	36	136	15	2	17	16	2	18				
Turdus chrysolaus	4	35	39	2	17	19	2	26	28				
Tarsiger cyanurus	0	17	17	0	5	5	0	5	5				
Emberiza variabilis	4	10	14	1	4	5	4	9	13				
Luscinia calliope	6	2	8	3	1	4	6	2	8				
Sitta europaea	3	0	3	1	0	1	1	0	1				
Troglodytes troglodytes	0	3	3	0	2	2	0	4	4				
Erithacus akahige	0	1	1	0	1	1	0	3	3				
Phylloscopus schwarzi	1	0	1	1	0	1	2	0	2				
Turdus obscurus	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

	No. capt	ured birds		No. birds in	fested by tic	ks*	No. ticks*					
Species of migratory bird	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total	Hamatonbetsu (Lake Kutcharo)	Nemuro (Lake Furen)	Total			
Emberiza	651	1,098	1,749	157	421	578	273	896	1,169			
spodocephala	001	1,030	1,745	157	421	570	215	030	1,103			
Uragus sibiricus	0	61	61	0	1	1	0	1	1			
Horornis	51	8	59	9	1	10	9	1	10			
diphone	51	0	55	5		10	5		10			
Turdus	8	24	32	4	20	24	7	37	44			
chrysolaus	0	24	52	4	20	24	1	57	44			
Emberiza	7	9	16	4	4	8	13	17	30			
variabilis	I	9	10	4	4	0	15	17	30			
	0	8	8	0	4	4	0	6	6			
Tarsiger	0	0	0	0	4	4	0	0	0			
cyanurus Certhia	2	4	6	1	1	2	1	1	2			
familiaris	Z	4	0	I	I	2	I	I	2			
	c	0	c	1	0	1	1	0	1			
Parus minor	6 0	0	6 6	1	0	1	1	0 7	7			
Troglodytes	0	6	ю	0	4	4	0	1	1			
troglodytes	0	-	-	0			0					
Parus palustris	0	5	5	0	1	1	0	1	1			
Luscinia	4	0	4	1	0	1	2	0	2			
calliope		-										
Sitta europaea	0	2	2	0	1	1	0	1	1			
Turdus	1	0	1	1	0	1	1	0	1			
obscurus												
Total	730	1,225	1955	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

				St	tatus of bl	oodfeeding
Strain	Tick species	Stage	No. ticks in pool	Engorged	Unfed	Engorged or unfed
YEZV/tick/BT-1821/Japan/2020	Ixodes persulcatus	Nymph	5	1	4	0
YEZV/tick/BT-1822/Japan/2020	Ixodes persulcatus	Nymph	5	1	4	0
YEZV/tick/BT-1826/Japan/2020	lxodes persulcatus	Nymph	5	0	5	0
YEZV/tick/BT-1844/Japan/2020	lxodes persulcatus	Larva	10	3	6	1
YEZV/tick/BT-1864/Japan/2020	Ixodes persulcatus	Larva	10	0	10	0
YEZV/tick/BT-1968/Japan/2020	lxodes persulcatus	Larva	10	3	1	6
YEZV/tick/BT-2135/Japan/2021	Ixodes persulcatus	Nymph	5	0	5	0
YEZV/tick/BT-2155/Japan/2021	Ixodes persulcatus	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*

								YEZV/hu man/HH0	YEZV/hu man/HH0	YEZV/hu man/HH0	YEZV/hu man/HH0	YEZV/hu man/HH0	YEZV/hu man/HH0				YEZV/tick	YEZV/tick/			YEZV/tick/	YEZV/tick	Sulina
	YEZV/tick /BT-	YEZV/tick /BT-	YEZV/tick/ BT-	YEZV/tick /BT-	YEZV/tick /BT-	YEZV/tick/ BT-	YEZV/tick /BT-	man/HHU 01-	man/HHU 03-	man/HH0 07-2016	man/HHU 08-	man/HHU 09-	man/HHU 11-	YEZV/hu man/H-	YEZV/tick/ T-	YEZV/tick /T-	YEZV/tick /T-	TIGMIC 1	YEZV/tick /TIGMIC	YEZV/tick /TIGMIC	YEZV/tick/	YEZV/tick /T-JL01	Sulina virus
	1821/Jap	1826/Jap	1844/Jap	1864/Jap	1968/Jap	2135/Jap	2155/Jap	2019/Jap	2020/Jap	/Japan/20	2017/Jap	2017/Jap	2020/Jap	IM01/Chin	-		HLJ03/Ch	/China/20	2/China/2	3/China/2	IM01/Chin	/China/20	IxriSL16-
Virus	an/2020	an/2020	an/2020	an/2020	an/2020	an/2021	an/2021	an/2019	an/2020	16	an/2017	an/2017	an/2020	a/2018	ina/2021	ina/2021	ina/2021	19	019	019	a/2021	20	01
YEZV/tick/BT-	_	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
1821/Japan/2020																							
YEZV/tick/BT-	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
1826/Japan/2020																							
YEZV/tick/BT-	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
1844/Japan/2020																							
YEZV/tick/BT-	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
1864/Japan/2020																							
YEZV/tick/BT-	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
1968/Japan/2020	00 7	00 7	~~~~		~~~~		00.4	07.4	<u> </u>	07 5		07.4	07.5	<u> </u>	00 7	00 F	~~~~	~~~~	00 F	~~~~		~~~~	70.0
YEZV/tick/BT-	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
2135/Japan/2021 YEZV/tick/BT-	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	09.6	98.4	98.8	98.8	98.5	98.5	98.3	98.8	72.8
2155/Japan/2021	99.9	99.9	100.0	99.5	99.0	99.0	_	97.5	99.1	97.4	90.0	97.2	97.4	90.4	98.6	90.4	90.0	90.0	90.5	90.5	90.5	90.0	12.0
YEZV/human/HH001-	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
2019/Japan/2019	00.0	00.0	00.0	00.0	00.0	00.1	00.0		07.4	00.4	07.2	00.0	00.4	07.4	07.2	07.4	07.4	07.2	01.4	01.0	07.0	07.0	72.0
YEZV/human/HH003-	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
2020/Japan/2020																							
YEZV/human/HH007-	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
2016 /Japan/2016																							
YEZV/human/HH008-	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
2017/Japan/2017																							
YEZV/human/HH009-	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
2017/Japan/2017	00 F	<u> </u>	<u> </u>	00.4	00 7	00 7	~~~~	<u> </u>	~~~~	400.0	<u> </u>	oo 7		07.5	07.0	07.5	07 5	07.4	07.0	07.0	07.4	07.4	70.0
YEZV/human/HH011-	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
2020/Japan/2020 YEZV/human/H-	00 5	00 F	99.6	00 F	99.7	00.7	99.6	99.7	00.6	99.6	99.6	99.5	99.6		98.2	98.5	98.4	98.3	09 5	09.6	100.0	00.2	70.4
IM01/China/2018	99.5	99.5	99.0	99.5	99.7	99.7	99.0	99.7	99.6	99.0	99.0	99.5	99.0	_	90.2	96.5	90.4	90.3	98.5	98.6	100.0	98.3	72.4
YEZV/tick/T-	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
HLJ01/China/2021	55.0	55.0	55.0	55.5	55.5	55.0	55.7	55.7	55.0	55.0	55.0	55.5	55.0	55.7		50.5	50.5	50.0	50.4	50.4	50.2	50.0	72.0
YEZV/tick/T-	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
HLJ02/China/2021																							
YEZV/tick/T-	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
HLJ03/China/2021																							
YEZV/tick/TIGMIC_1/C	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
hina/2019																							
YEZV/tick/TIGMIC_2/C	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
hina/2019	~~ -	~~ -									~~ -										~~ -	~~ -	
YEZV/tick/TIGMIC_3/C	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
hina/2019 YEZV/tick/T-	00 F	00 5	00.6	00 5	00.7	00.7	00.6	00.7	00.6	00.6	00.6	00 5	00.6	100.0	00.7	00.7	00.6	00.7	00.9	00.9		00.2	70 /
YEZV/tick/1- 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	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
/China/2020	33.5	33.5	33.0	33.4	33.0	33.1	33.0	33.0	33.1	33.5	33.5	53.4	33.5	33.0	53.0	55.0	33.0	33.1	33.1	55.1	33.0		12.0
Sulina virus IxriSL16-	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	82.0	82.1	_
01	02.1	02.2	02.2	02.0	02.1	02.1	02.1	02.2	02.1	02.2	02.1	02.1	52.2	02.0	02.2	02.1	02.1	02.1	02.1	02.1	02.0	02.1	

*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*

										YEZV/huma										
	YEZV/tick/B			YEZV/tick/B			YEZV/tick/B			n/HH007-	YEZV/huma				YEZV/tick/T	YEZV/tick/T	YEZV/tick/T	YEZV/tick/T		
	T-	T-	T-	T-	T-	T-	T-	n/HH001-	n/HH003-	2016	n/HH008-	n/HH009-	n/HH011-	n/H-	-	-	-	-	YEZV/tick/T	o
<i>\ C</i>	1821/Japan	1826/Japan	1844/Japan	1864/Japan	1968/Japan	2135/Japan	2155/Japan	2019/Japan		/Japan/201	2017/Japan	2017/Japan	2020/Japan	IM01/China/	HLJ01/Chin	HLJ02/Chin	HLJ03/Chin	IM01/China/	-JL01	Sulina virus
Virus	/2020	/2020	/2020	/2020	/2020	/2021	/2021	/2019	/2020	6	/2017	/2017	/2020	2018	a/2021	a/2021	a/2021	2021	/China/2020	
YEZV/tick/BT-	_	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
1821/Japan/2020	00.0		00 7	00.4	00.4	07.5	00.7	07.0	00.0	07.5	00.0	07.4	07.5	00.0	00.7	00.0	00.7	00.0	00.0	00.0
YEZV/tick/BT-	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
1826/Japan/2020	00.0	00.0		00.4	00 5	07 5	100.0	07.0	00.0	07.5	99.3	07.0	07.5	98.3	98.7	00.0	98.7	98.3	98.7	<u> </u>
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-	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
1864/Japan/2020	99.0	99.9	99.0	_	99.5	97.0	99.4	96.0	99.5	97.0	99.0	97.5	97.0	90.5	90.9	90.7	90.0	90.0	90.0	09.1
YEZV/tick/BT-	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
1968/Japan/2020	99.5	99.1	99.0	99.0	_	97.5	99.0	97.9	99.2	97.5	99.5	91.2	97.5	90.5	90.7	90.0	90.0	90.5	90.0	09.0
YEZV/tick/BT-	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
2135/Japan/2021	33.2	33.4	33.5	33.5	33.2	_	51.5	33.0	51.5	30.5	57.0	30.0	30.5	57.5	51.5	51.1	57.4	57.5	57.4	03.0
YEZV/tick/BT-	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
2155/Japan/2021	00.0	00.0	00.0	00.1	00.1	00.0		07.0	00.2	01.0	00.0	01.2	01.0	00.0	00.1	00.0	00.7	00.0	00.1	00.0
YEZV/human/HH001-	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
2019/Japan/2019	00.1	00.0	00.0	00.0	00.1	00.0	00.0		01.0	00.1	01.0	00.0	00.1	01.0	01.0	00.0	01.1	01.0	01.1	00.1
YEZV/human/HH003-	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
2020/Japan/2020										••••		••••	••••							
YEZV/human/HH007-	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
2016 /Japan/2016																				
YEZV/human/HH008-	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
2017/Japan/2017																				
YEZV/human/HH009-	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
2017/Japan/2017																				
YEZV/human/HH011-	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
2020/Japan/2020																				
YEZV/human/H-	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
IM01/China/2018																				
YEZV/tick/T-	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
HLJ01/China/2021																				
YEZV/tick/T-	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
HLJ02/China/2021																				
YEZV/tick/T-	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
HLJ03/China/2021																				
YEZV/tick/T-	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
IM01/China/2021	~~ -						~~~~	~~ -		~~ -		<u> </u>				~~~~				aa (
YEZV/tick/T-JL01	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
/China/2020	57.0	F7 4	F7 4	F7 0	57.0	F7 0	F7 4	F7 4	F7 4	F7 4	F7 0	57.0	F7 4	57.0	F7 4	F7 4	57.0	57.0	F7 4	
Sulina virus IxriSL16-	57.0	57.1	57.1	57.0	57.2	57.0	57.1	57.1	57.1	57.1	57.0	57.3	57.1	57.3	57.1	57.1	57.0	57.3	57.1	—
01																				

*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*

										YEZV/hum		YEZV/hu								
	YEZV/tick/	YEZV/hum	YEZV/hum	an/HH007-	YEZV/hum	man/HH00	YEZV/hum	YEZV/huma	YEZV/tick	YEZV/tick/	YEZV/tick/	YEZV/tick/	YEZV/tick/							
	BT-	an/HH001-	an/HH003-	2016	an/HH008-	9-	an/HH011-	n/H-	/T-	Т-	Т-	Т-	T-JL01	Sulina						
	1821/Japa	1826/Japa	1844/Japa	1864/Japa	1968/Japa	2135/Japa	2155/Japa	2019/Japa	2020/Japa	/Japan/201	2017/Japan	2017/Japa	2020/Japa	IM01/China/	HLJ01/Ch	HLJ02/Chi	HLJ03/Chi	IM01/China	/China/202	virus
Virus	n/2020	n/2020	n/2020	n/2020	n/2020	n/2021	n/2021	n/2019	n/2020	6	/2017	n/2017	n/2020	2018	ina/2021	na/2021	na/2021	/2021	0	IxriSL16-01
YEZV/tick/BT	—	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
-																				
1821/Japan/2 020																				
YEZV/tick/BT	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
-																				
1826/Japan/2																				
020																				
YEZV/tick/BT	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
- 1844/Japan/2																				
020																				
YEZV/tick/BT	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
-																				
1864/Japan/2																				
020																				

Virus	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/hum an/HH001- 2019/Japa n/2019	YEZV/hum an/HH003- 2020/Japa n/2020	YEZV/hum an/HH007- 2016 /Japan/201 6	YEZV/hum an/HH008- 2017/Japan /2017	YEZV/hu man/HH00 9- 2017/Japa n/2017	YEZV/hum an/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/202 0	Sulina virus IxriSL16-01
YEZV/tick/BT	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
- 1968/Japan/2 020 YEZV/tick/BT	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
- 2135/Japan/2 021	00.0	100.0	100.0	100.0	400.0	100.0		00.7	00.0	00.0	00.4	00.0	00.0	00.0	00.0	00.0	00.7	00.0	00.0	00.0
YEZV/tick/BT - 2155/Japan/2 021	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/2	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
019 YEZV/human /HH003- 2020/Japan/2	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
020 YEZV/human /HH007-2016	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
/Japan/2016 YEZV/human /HH008- 2017/Japan/2 017	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/2 017	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/2 020	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/2	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
018 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/2 021	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.