Wild Mice and Shrews as Natural Reservoirs of Emerging Tickborne Yezo Virus, Hokkaido, Japan, 2023–2025

Mebuki Ito, Yuma Ohari, Isshu Kojima,[1] Yume Mimura, Keita Mizuma,[2] Jingshu Li, Hayato Torisu, Shumpei Kamiyama, Tomoki Sakiyama,[3] Hironori Mieda, Naoya Matsumoto, Natsumi Iijima, Eri Fujii, Yasuko Orba, Kuniko Kawai, Satoshi D. Ohdachi, Keita Matsuno

We screened 420 wild small mammals and 2,273 ticks for infections with an emerging tickborne orthonairovirus, Yezo virus, during 2023–2025 in Hokkaido, Japan. We detected virus in mice, shrews, and ticks that were closely related. *Apodemus* spp. mice and *Sorex* spp. shrews were identified as potent natural reservoirs of Yezo virus.

Yezo virus (YEZV) is an emerging tickborne orthonairovirus initially identified in febrile patients after a tick bite in Hokkaido, Japan (*1*). Since the first report in 2021, YEZV infections in humans have been reported from Hokkaido, northeastern China, and Russia (*2*–*5*). Despite its public health significance, our understanding of YEZV circulation in nature remains limited. In previous studies (*1*,*6*,*7*), we demonstrated that raccoons (*Procyon lotor*), tanukis (*Nyctereutes procyonoides*), and sika deer (*Cervus nippon*) possess antibodies against YEZV, suggesting YEZV circulation in the field. However, the vertebrate reservoirs remain unidentified. In contrast, among several tick species in YEZV-endemic regions, *Ixodes* spp. ticks are suspected to play crucial roles in the pathogen's circulation and transmission to humans (*3*,*7*–*9*). Given that larval and nymphal *Ixodes* spp. ticks mainly infest small mammals in Hokkaido (*10*), we hypothesized that those animals serve as natural reservoir hosts of YEZV. This study reports YEZV infection status and the phylogenetic relationships of YEZV in 3 taxonomic groups of native small mammals in Hokkaido: mice (order Rodentia, family Muridae, genus *Apodemus*), voles (order Rodentia, family Cricetidae, genus *Craseomys* and *Clethrionomys*), and shrews (order Eulipotyphla, family Soricidae, genus *Sorex*) (*11*,*12*).

The Study

We captured small mammals at 14 sites across Hokkaido in 2023 and 2025 (Appendix Figure, https://wwwnc.cdc.gov/EID/article/32/12/26-0298-App1.pdf). We identified the species of animal on the basis of morphologic features (*11*) and confirmed species by sequencing the mitochondrial genome (data not shown). We collected liver, spleen, lung, heart, kidney, brain, and serum samples of trapped animals. All animal experiments were approved by the Animal Care and Use Committee of Hokkaido University (approval nos. 23-0063, 23–0178, and 25-0119). Permission to capture wild mammals was obtained from the Hokkaido Government.

We captured a total of 420 wild small mammals, consisting of 252 mice (*Apodemus argenteus* and *A. speciosus*), 121 voles (*Craseomys rufocanus* and *Clethrionomys rutilus*), and 47 shrews (*Sorex caecutiens*, *S. gracillimus*, and *S. unguiculatus*). We collected serum samples from 199 animals and screened them for YEZV-specific antibodies by ELISA (*1*). Of the 199 serum samples, 14 (7.0%) samples were positive

Author affiliations: Hokkaido University International Institute for Zoonosis Control, Sapporo, Japan (M. Ito, Y. Ohari, I. Kojima, Y. Mimura, K. Mizuma, J. Li, E. Fujii, Y. Orba, K. Matsuno); Hokkaido University Graduate School of Environmental Science, Sapporo (H. Torisu, T. Sakiyama, H. Mieda); Tokai University Graduate School of Biology, Sapporo (S. Kamiyama, K. Kawai); Sapporo Maruyama Zoo, Sapporo (N. Matsumoto, N. Iijima); Hokkaido University Institute of Low Temperature Science, Sapporo (S.D. Ohdachi)

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[1]Current affiliation: Okayama University of Science Faculty of Veterinary Medicine, Imabari, Japan.

[2]Current affiliation: Tokyo University, Tokyo, Japan.

[3]Current affiliation: Umeå University, Umeå, Sweden.

Table 1. Screening for infection in mice, voles, and shrews in study of wild mice and shrews as natural reservoirs of emerging tickborne Yezo virus, Hokkaido, Japan, 2023–2025*

Taxonomic group	Species	No. ELISA-positive/no. tested (%)	No. real-time qRT-PCR–positive/no. tested (%)
Mouse	*Apodemus argenteus*	3/78 (3.8)	6/122 (4.9)
	A. speciosus	8/61 (13.1)	5/130 (3.8)
Vole	*Craseomys rufocanus*	2/53 (3.8)	0/113 (0.0)
	Clethrionomys rutilus	1/6 (17)	0/8 (0.0)
Shrew	*Sorex caecutiens*	NA	1/11 (9.1)
	S. gracillimus	NA	3/19 (15.8)
	S. unguiculatus	0/1 (0)	1/17 (5.9)
Total		14/199 (7.0)	16/420 (3.8)

*NA, not applicable; qRT-PCR, quantitative reverse transcription PCR.

for YEZV antibodies (Table 1). The seroprevalence was not statistically different between mice and voles (p = 0.561 by Fisher exact test). We screened liver samples from all 420 animals for YEZV small (S) segment RNA using real-time quantitative reverse transcription PCR (qRT-PCR) (*8*). We detected YEZV RNA in 16 samples from mice and shrews, whereas all voles tested negative (Table 1). RNA detection rates in mice and shrews were significantly higher than those in voles (p = 0.038 [mice] and p = 0.004 [shrews] by pairwise comparison using Fisher exact test with Holm adjustment). Among the 199 individual animals tested by both real-time qRT-PCR and ELISA, 6 were RNA-positive/antibody-negative, 4 were RNA-positive/antibody-positive, and 10 were RNA-negative/antibody-positive, reflecting different stages of infection.

We further quantified the amount of S, medium (M), and large (L) segment RNA in the 16 viral RNA-positive samples using the following primers

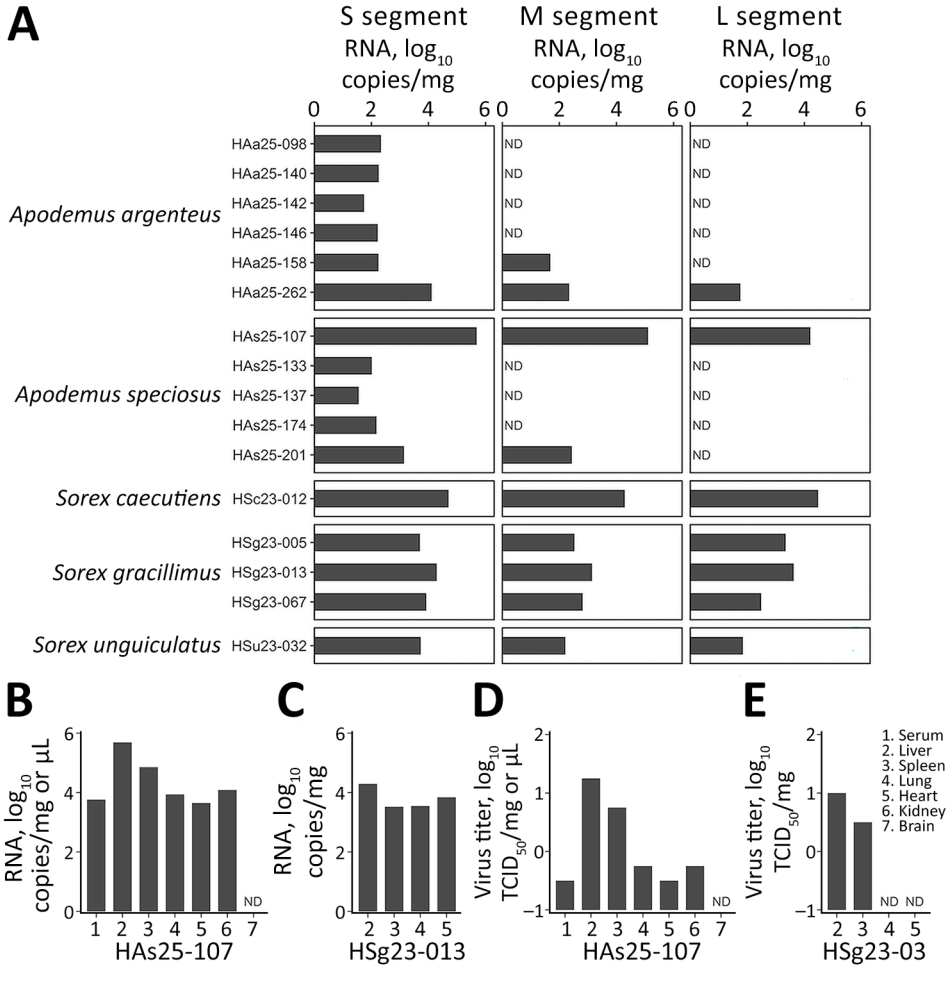


Figure 1. RNA copy numbers and viral titers in organs of wild small mammals in study of wild mice and shrews as natural reservoirs of emerging tickborne Yezo virus, Hokkaido, Japan, 2023–2025. A) Copy numbers of 3 segments in the livers of wild small mammals. Liver samples positive for the S segment were used for this analysis. The detection limits were 1.62 $\log_{10}$ copies/mg for M segment and 1.52 $\log_{10}$ copies/mg for L segment. B–E) RNA copy numbers and viral titers of the serum and internal organs quantified using the 2 animals from which the virus was isolated (HAs25-107 and HSg23-013). B, C) Copy numbers of the S segment RNA quantified by quantitative reverse-transcription PCR. D, E) $TCID_{50}$ determined using ISE6 cells (*Ixodes scapularis* embryo–derived cells), which was provided by the Centre for Ecology and Hydrology Institute of Virology and Environmental Microbiology (Oxford, UK) (*14*). The detection limits of virus titer were −0.5 $\log_{10}$ $TCID_{50}$/mg for the liver, lung, and heart of HSg23-013; −2.5 $\log_{10}$ $TCID_{50}$/µL for serum of HAs25-107; and −1.5 $\log_{10}$ $TCID_{50}$/mg for the other samples. Serum, kidney, and brain samples of HSg23-013 were not available. L, large; M, medium; ND, not detected; S, small; $TCID_{50}$, 50% tissue culture infectious dose.

Table 2. Antibody detection, virus isolation, and viral RNA sequencing of small mammals and ticks positive for Yezo virus RNA in study of wild mice and shrews as natural reservoirs of emerging tickborne Yezo virus, Hokkaido, Japan, 2023–2025*

Species (no. in the pool)	Animal ID (isolate)	Site ID	Collection date	ELISA result	Isolation	Accession nos.†		
						S	M	L
Apodemus argenteus	HAa25-098	C104	2025 May 30	Serum not available	Fail	ND	ND	ND
	HAa25-140	C112	2025 Jun 3	Negative	Fail	ND	ND	ND
	HAa25-142	C112	2025 Jun 3	Negative	Fail	PX939685‡	ND	ND
	HAa25-146	C112	2025 Jun 3	Negative	Fail	ND	ND	ND
	HAa25-158	C112	2025 Jun 3	Positive	Fail	ND	ND	ND
	HAa25-262	C140	2025 Jun 14	Positive	Fail	PX939687	PX939686	ND
A. speciosus	HAs25-107	C112	2025 Jun 2	Negative	Success	PX939690	PX939689	PX939688
	HAs25-133	C112	2025 Jun 3	Negative	Fail	ND	ND	ND
	HAs25-137	C112	2025 Jun 3	Negative	Fail	ND	ND	ND
	HAs25-174	C013	2025 Jun 6	Positive	Fail	ND	ND	ND
	HAs25-201	C047	2025 Jun 10	Positive	Fail	PX939691	ND	ND
Sorex caecutiens	HSc23-012	W1	2023 Jun 27	Serum not available	Fail	PX939701	PX939700	PX939699
S. gracillimus	HSg23-005	W1	2023 Jun 25	Serum not available	Fail	PX939703	ND	PX939702
	HSg23-013	W1	2023 Jun 27	Serum not available	Success	PX939706	PX939705	PX939704
	HSg23-067	W2	2023 Jul 27	Serum not available	Fail	PX939709	PX939708	PX939707
S. unguiculatus	HSu23-032	W2	2023 Jul 25	Serum not available	Fail	ND	ND	ND
Ixodes ovatus (10 adults)	HIov25-157	C047	2025 Jun 17	NA	NA	ND	ND	ND
I. ovatus (8 adults)	HIov25-206	C140	2025 Jun 18	NA	NA	PX939694‡	ND	ND
I. persulcatus (3 adults)	HIpe24-390§	C047	2024 Jun 6	NA	NA	PX939697	PX939696	PX939695
I. persulcatus (2 adults)	HIpe25-146	C033	2025 Jun 17	NA	NA	ND	ND	ND
I. persulcatus (1 adult)	HIpe25-192	C104	2025 Jun 18	NA	NA	PX939698	ND	ND
Haemaphysalis flava (1 adult)	HHfl25-046	C071	2025 May 13	NA	NA	PX939692‡	ND	ND
Ixodes spp. (14 nymph)	HIN25-100	C033	2025 May 13	NA	NA	ND	ND	ND
Haemaphysalis spp. (5 nymph)	HHN25-212	C089	2025 Jul 8	NA	NA	PX939693‡	ND	ND

*L, large; M, medium; NA, not applicable; ND, sequence not determined; S, small.
†The sequences of isolates HAs25-107 and HSg23-013 were obtained from the liver and serum samples of AG129 mice inoculated with the liver samples of the wild mammals. The other isolates were directly sequenced from the original liver samples of the wild small mammals.
‡Partial sequence.
§HIpe24-390 was identified in our previous study (*8*).

and probes: for the M segment, forward primer 5′-TGGAGTCAAGGGCTGTTATG-3′, reverse primer 5′-CACCAGGCATTTACCTCTACTT-3′, and probe 5′-/56-FAM/TAAGCAGGC/ZEN/TGACATGGAGCCTTT/3IABkFQ/-3′; for the L segment, forward primer 5′-CTCAACCTGCTTCCAACCTATC-3′, reverse primer 5′-GGTGTAAAGCCCAACATCCT-3′, and probe 5′-/5Cy5/CCAAGGAAG/TAO/CACACAGATGGGTACA/3IAbRQSp/-3′. The copy numbers of the S segment RNA were consistently higher than those of the M and L segment RNA (Figure 1, panel A). Of note, all 5 shrews tested positive for all 3 segments, whereas only 2 of the 11 mice were positive for all 3 segments, suggesting that the mode of viral replication differs between mice and shrews.

To confirm production of infectious virions, we attempted virus isolation using the 16 liver samples that tested positive for viral RNA (Table 2). We intraperitoneally inoculated AG129 mice, which showed higher susceptibility to YEZV infection than did cell lines (*1*,*4*), with each sample. Subsequently, we inoculated the serum and liver samples of AG129 mice to ISE6 cells. Finally, we successfully isolated YEZV from liver samples from HAs25-107 (*A. speciosus* mouse) and HSg23-013 (*S. gracillimus* shrew). To investigate the systemic infection and tissue tropism of YEZV in those wild mammals, we quantified RNA copy numbers and infectious titers in the serum and internal organs (Figure 1, panels B–E). We detected viral RNA in all examined organs except for the brain. Consistent with the RNA copy numbers, we did not detect infectious virus in the serum of HAs25-107 and multiple organs of both animals; the highest virus titers and RNA copy numbers were in the liver.

In 2025, ticks were also collected by a flagging method at the same sites where small mammals were

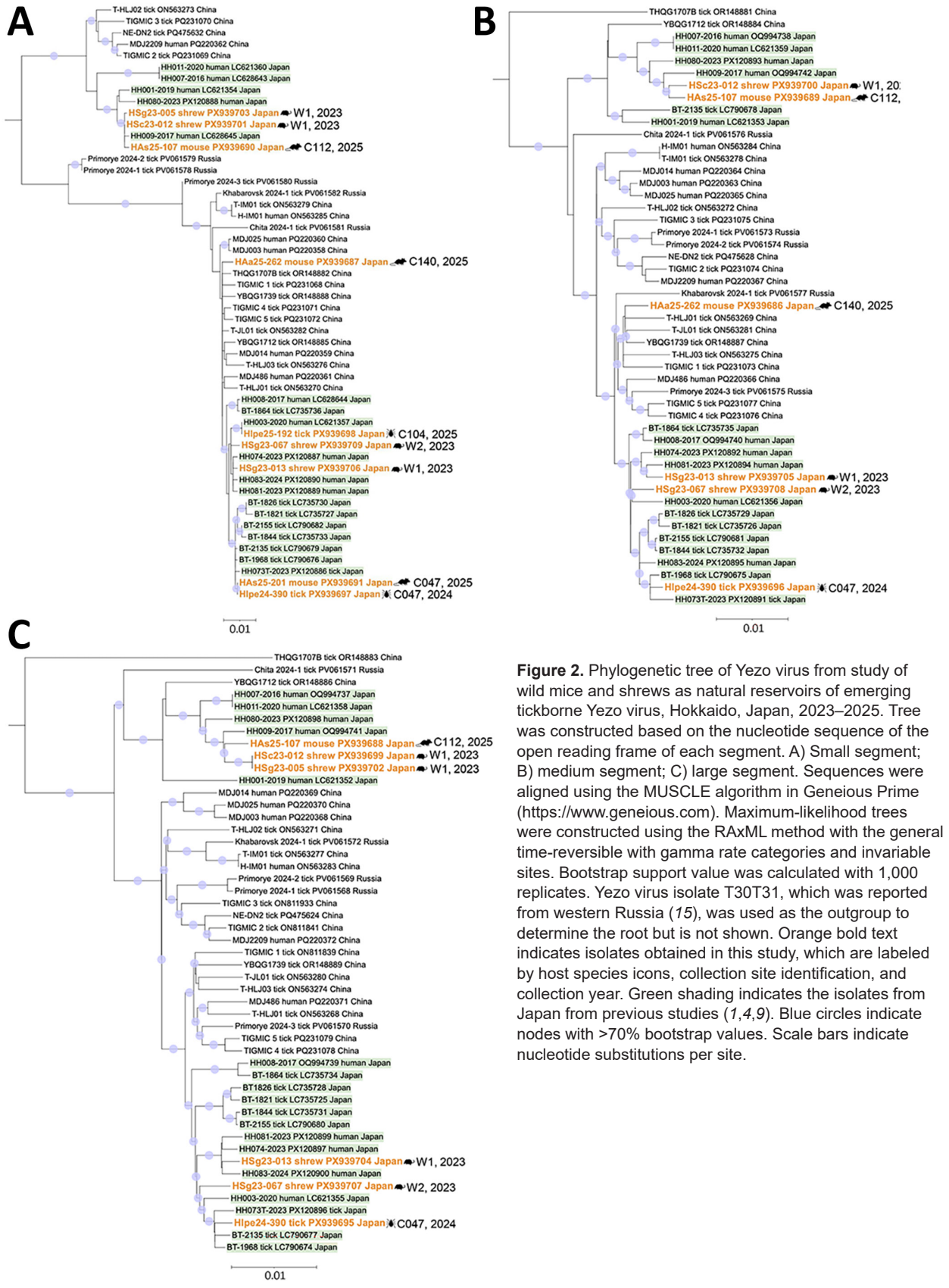


Figure 2. Phylogenetic tree of Yezo virus from study of wild mice and shrews as natural reservoirs of emerging tickborne Yezo virus, Hokkaido, Japan, 2023–2025. Tree was constructed based on the nucleotide sequence of the open reading frame of each segment. A) Small segment; B) medium segment; C) large segment. Sequences were aligned using the MUSCLE algorithm in Geneious Prime (https://www.geneious.com). Maximum-likelihood trees were constructed using the RAxML method with the general time-reversible with gamma rate categories and invariable sites. Bootstrap support value was calculated with 1,000 replicates. Yezo virus isolate T30T31, which was reported from western Russia (*15*), was used as the outgroup to determine the root but is not shown. Orange bold text indicates isolates obtained in this study, which are labeled by host species icons, collection site identification, and collection year. Green shading indicates the isolates from Japan from previous studies (*1*,*4*,*9*). Blue circles indicate nodes with >70% bootstrap values. Scale bars indicate nucleotide substitutions per site.

captured for this study to examine local transmission cycles. Of the 2,286 ticks collected, 2,273 ticks were pooled into 288 samples and screened by real-time qRT-PCR (*8*). A total of 7 pools tested positive for YEZV RNA (Table 2).

To examine the relationship among YEZV infections in mammals and ticks, we determined complete coding sequences for the S (n = 7), M (n = 5), and L (n = 5) segments from small mammal samples and for the S (n = 2), M (n = 1), and L (n = 1) segments for *Ixodes persulcatus* tick pools (Table 2). The sequences of YEZV isolates obtained from small mammals and questing ticks in this study shared high identity with previously reported sequences from human patients and ticks collected from humans and birds in Japan (93.2%–100.0% at the nucleotide level and 99.0%–100.0% at the amino acid level) (Appendix Tables 1–3). Phylogenetic analysis based on those sequences demonstrated that most YEZV isolates from small mammals and ticks clustered with isolates from Japan (Figure 2). Some sequences from the same sampling sites (i.e., isolates HSg23-005 and HSc23-012 and isolates HIpe24-390 and HAs25-201) formed site-specific clusters with high identity (>99.9% at the nucleotide level) (Appendix Tables 1, 3). In contrast, isolates HSc23-012 and HSg23-013 from the same site belong to phylogenetically distinct clusters (Table 2; Figure 2). One isolate (HAa25-262) from an *A. argenteus* mouse clustered with isolates reported in China rather than with isolates from Japan.

Conclusions

This study identified *Apodemus* spp. mice and *Sorex* spp. shrews as natural reservoir hosts for YEZV and confirmed local circulation of the virus in Hokkaido, Japan. Systemic infection with viremia indicates that wild mice and shrews serve as a source of infection for feeding ticks, which might subsequently transmit the virus to humans. In contrast, all the liver samples of voles (*Cr. rufocanus* and *Cl. rutilus*) were negative for viral RNA despite showing seropositivity comparable to mice, implying that voles contribute less to YEZV circulation. The cocirculation of the diverse isolates at local sites suggests the frequent movement of YEZV within Hokkaido as well as between Hokkaido island (Japan) and Eurasia (China and Russia). Given that wild mice and shrews have small home ranges (*13*), YEZV is likely dispersed by highly mobile species, such as migratory birds (*8*,*9*). In addition, because those shrew and mouse species and their close relatives, together with *Ixodes* ticks, are also distributed in areas considered as nonendemic for YEZV, such as the Korean Peninsula and the main island of Japan (Honshu) (*11*,*12*), YEZV might be more widely distributed than currently recognized. Our findings provide critical insights into the ecology of YEZV and highlight the need for efficient epidemiologic surveillance for this virus.

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About the Author

Mr. Ito is a PhD student in the Division of Risk Analysis and Management, International Institute for Zoonosis Control, Hokkaido University. His primary research interest is the ecology of tickborne viruses.

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Address for correspondence: Keita Matsuno, Division of Risk Analysis and Management, International Institute for Zoonosis Control, Hokkaido University, Kita 20, Nishi 10, Kita-ku, Sapporo City 001-0020, Hokkaido, Japan; email: matsuk@czc.hokudai.ac.jp

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