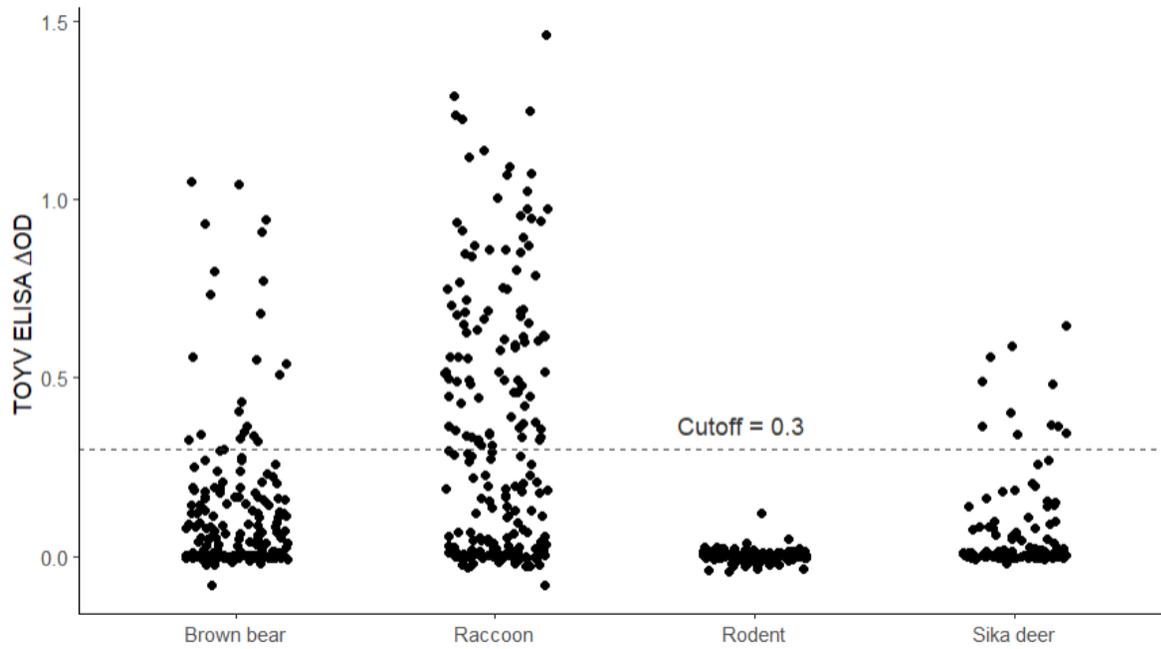


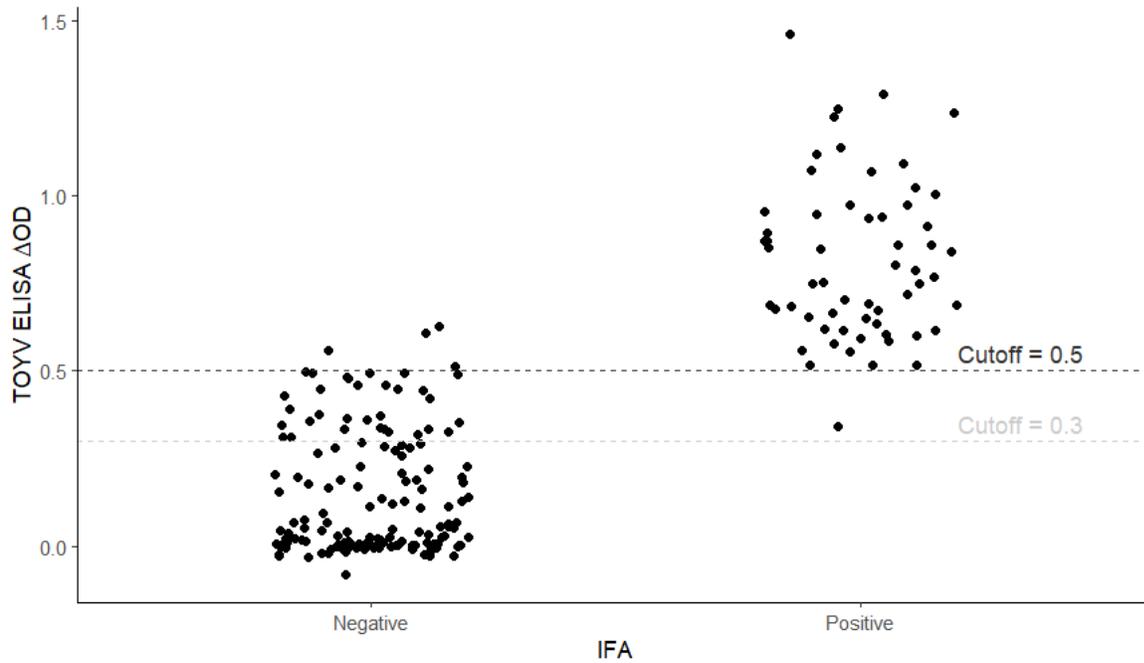
1 **Supplementary figures**



2

3 Supplementary Figure 1. ELISA for TOYV infections in wild animals in Hokkaido. OD ratios
4 were calculated as OD of the antigen-coated well minus the OD of the negative-control well. The
5 positive control was a lysate of TOYV-infected Hep3B cells, and the negative control was a
6 lysate of uninfected cells.

7



8

9 Supplementary Figure 2. TOYV ELISA cutoff optimization for raccoon samples based on IFA
10 results. ELISA OD ratios were calculated as OD of the antigen-coated well minus the OD of the
11 negative-control well. The positive control was a lysate of TOYV-infected Hep3B cells, and the
12 negative control was a lysate of uninfected cells. The x-axis indicates the IFA result of each
13 serum sample at a 1:50 dilution. The initial cutoff value of 0.3, as well as the adjusted cutoff of
14 0.5 applied to raccoon samples, are indicated.

15

16 **Supplementary Tables**

17 Supplementary Table 1. Primer and probe used for RT-qPCR assay targeting TOYV.

Target	ID	Type	Sequence (5'→3')
S segment	B1217	Forward primer	TTCACTTGGTGTGGACAGAG
	B1218	Reverse primer	GAACCTGGATGAGCTGCTAAA
	Q017_ToyoNS_P	Probe	FAM-CAGCCAGCATTGGCGAAAGTTGAG-TAMRA

18

19 Supplementary Table 2. Accession numbers of determined viral sequences.

Strain	Host	Collection date	Collection location	Accession number (GenBank)		
				S segment	M segment	L segment
23B51	<i>Ursus arctos yesoensis</i>	October, 2023	Shiretoko peninsula, Hokkaido, Japan	PX874832	PX874829	PX874823
23B53	<i>Ursus arctos yesoensis</i>	October, 2023	Shiretoko peninsula, Hokkaido, Japan	PX874835	PX874826	PX874820
23B55	<i>Ursus arctos yesoensis</i>	October, 2023	Shiretoko peninsula, Hokkaido, Japan	PX874833	PX874830	PX874825
23B65	<i>Ursus arctos yesoensis</i>	October, 2023	Shiretoko peninsula, Hokkaido, Japan	PX874834	PX874831	PX874824
23B72	<i>Ursus arctos yesoensis</i>	October, 2023	Shiretoko peninsula, Hokkaido, Japan		PX874828	PX874822
23B73	<i>Ursus arctos yesoensis</i>	October, 2023	Shiretoko peninsula, Hokkaido, Japan	PX874836	PX874827	PX874821
15THM	<i>Haemaphysalis megaspinosa</i>	April, 2015	Mukawa town, Hokkaido, Japan	PX874841	PX874839	PX874837
18THF	<i>Haemaphysalis flava</i>	October, 2018	Shiraoi town, Hokkaido, Japan	PX874842	PX874840	PX874838

21 Supplementary Table 3. Accession numbers of sequences used in this study.

Virus	Accession number		
	L segment	M segment	S segment
Toyo virus	NC_079057	NC_079058	NC_079059
Silverwater virus	KM114257	KM114255	KM114256
Kaisodi virus	MG581739	MG581740	MG581741
Khasan virus	KF892046	KF892047	KF892048
Huangpi Tick virus 2	KM817668	KM817707	KM817735
Precarious point virus	HM566181	HM566179	HM566180
Chize virus	JF838324	JF838325	JF838326
FinV707 virus	JQ924562	JQ924563	JQ924564
Zaliv Terpenia virus	NC_055356	NC_055354	NC_055355
Uukuniemi virus	NC_005214	NC_005220	NC_005221
Komandory virus	KF892049	KF892050	KF892051
Rukutama virus	NC_055368	NC_055367	NC_055366
Kabuto mountain virus	NC_036604	NC_036605	NC_036606
Gouleako virus	NC_043051	NC_043049	NC_043050

23 Supplementary Table 4. Brown bears positive for TOYV in 2023.

ID	Collect ion site	Sex	Weigh t	Age	Collection date (y/m/d)	Closest RefSeq relative (GenBank accession number)	% nucleoti de identity	Sequence
23B51		F	137.6	≥1 y.o.	2023/10/02		97.7	TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGGCACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTACTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGACACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTGCTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA
23B53		F	106.2	≥1 y.o.	2023/10/02		97.7	TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGACACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTGCTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA
23B55		F	96.7	≥1 y.o.	2023/10/03		97.7	TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGGCACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTACTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA
23B61		F	101.2	≥1 y.o.	2023/10/07		88.0*	TAITGANTAGGTTTTGAAGAGAGAGTTGTGCAGNTAGTAGNTGAAANGATATC CCGAACCAITTCAGGGCACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA AGCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG GCCATCAGNNCANTGGCANTNNANGATGCNCGACTTGGAAATCANGGTCA TCATGTGTCCAAGTCCCGNTGATNGTATTGATTGTTTACAAAAGANGAAGTGC ATCCTTTCTGNTCNGGGNTGGTANCTTTTCATGANGAACCGGATNATGCTAG ACCAGANNCTACTCANTATCCTTNAATTGAATCAAACCTNANANANANANGAC CCAACATTGAAACTGTTGCATGATGTTTACCATGGNAANGTGGNANTNGATTG NATGCCCAA
23B65	Shiret oko Peninsula, Shari	M	232.7	≥1 y.o.	2023/10/09	Toyo virus (NC_079057)	97.7	TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGGCACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTACTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA
23B72		M	27	≥1 y.o.	2023/10/12		97.7	TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGGCACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTACTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA
23B73		M	70	≥1 y.o.	2023/10/13		97.7	TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGACACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTGCTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA
23B74		F	102	≥1 y.o.	2023/10/15		97.7	TAITGACTAGGTTTTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATCC CGAACCAITTCGAGGCACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA GCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG CCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCATC ATGTGTCCAAGTCCCGCTGATGCTGATTCAGTTTACAAAAGATGAACCTGCATC CTTCTTGTTCAGGGCTGGTAGCTTTTCATGAGGAAGCGGATCATGCTAGACC AGAGGCTACTCAGTATCCTTGAATTGAATCAAACCTAGAAAACGAATGACCCA ACATTGAAACTGTTGCATGATGTTTACCATGGGAATGTGGCAGTTGATTGGATG CCCAA
23B98		F	81	≥1 y.o.	2023/11/11		89.2*	TAITGACTAGGTTGTGAAGAGAGAGTTGTGCAGCTAGTAGTTGAAACGATATC CCGAACCAITTCAGGGCACTTTCCTTCAGAAAACCTCACCATCCTAAGAGTAA ANCTGTCAAITCCTGAGTTGCATGGAAGGTATGCTTCAAAGATTGTGGATCCCG GCCATCAGACCATTGGCACATCAGATGATGCACGGACTTGGAAATCAGGGTCAT CATGTGTCCAAGTCCCGNTGATGCTGATTCAGTTTACAAAAGATGAACCTGCAT TCCCTTNTGTCAGGGCTGGTANCTTTTCATGANGAAGCANNATATGNCNG ANCAGAGNCTACNNNGTNTCTTNAANTGAATCAAACNNNNNNNNNNNANN ACCCAACNNNGAANNNTGTCNTGNNGTNNCCANNNNNAANGNNAGTNN ATNGNNNGCCCAA

24 *Sequences with nucleotide ambiguities.

25 y.o., year old.

26 Supplementary Table 5. TOYV RT-qPCR screening in ticks from the Shiretoko Peninsula (2024-
 27 2025).

Species	Sex	2024				2025				Total ticks	Total pools
		Spring-Summer		Autumn		Spring-Summer		Autumn			
		Tested tick number	Pools number								
<i>Ixodes ovatus</i>	F	17	4	0	0	13	3	0	0	30	7
	M	11	5	0	0	17	5	0	0	28	10
<i>Ixodes persulcatus</i>	F	10	3	0	0	4	1	0	0	14	4
	M	7	2	0	0	13	4	0	0	20	6
<i>Haemaphysalis japonica</i>	F	36	8	0	0	27	7	0	0	63	15
	M	13	4	4	2	26	7	0	0	43	13
<i>Haemaphysalis megaspinosa</i>	F	1	1	44	10	7	3	55	11	107	25
	M	0	0	34	10	1	1	55	11	90	22
Total		95	27	82	22	108	31	110	22	395	102

28

29 Supplementary Table 6. Follow-up TOYV RT-PCR screening in brown bears.

Location	Year	Capture season	Tested	Spleen TOYV RT-PCR		
				RT-PCR+	RT-PCR+ %	Total +/-tested (%)
West Shiretoko (Shari)	2023	April-August	28	0	0	9/89 (10.1)
		September-December	61	9	15	
	2024	April-August	15	0	0	0/15 (0.0)
		September-December	NA	NA	NA	
	2025*	April-August	22	0	0	4/40 (10.0)
		September-December	18	4	22	
East Shiretoko (Rausu)	2023	April-August	21	0	0	0/60 (0.0)
		September-December	39	0	0	
	2024	April-August	NA	NA	NA	0/4 (0.0)
		September-December	4	0	0	
	2025*	April-August	6	NA	NA	2/20 (10.0)
		September-December	14	2	14	

30 NA, not available.

31 *Samples tested with TOYV-specific RT-qPCR.