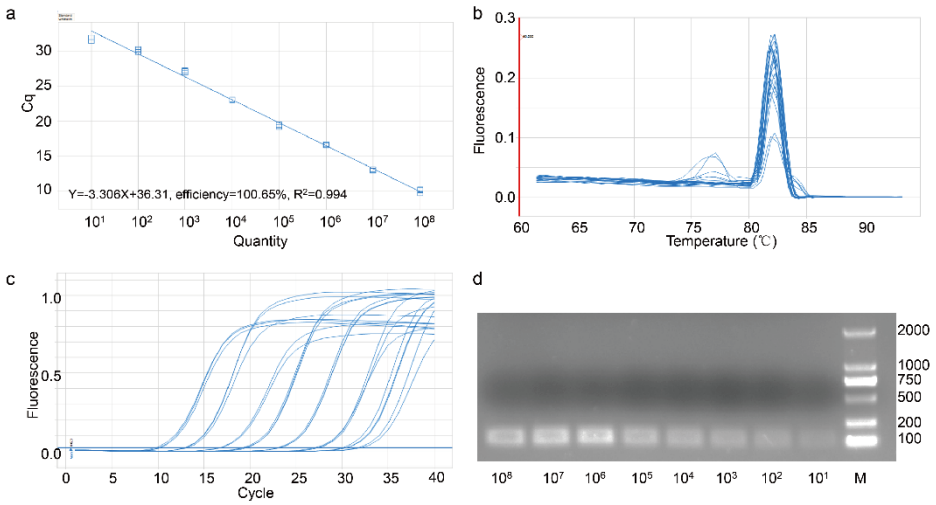
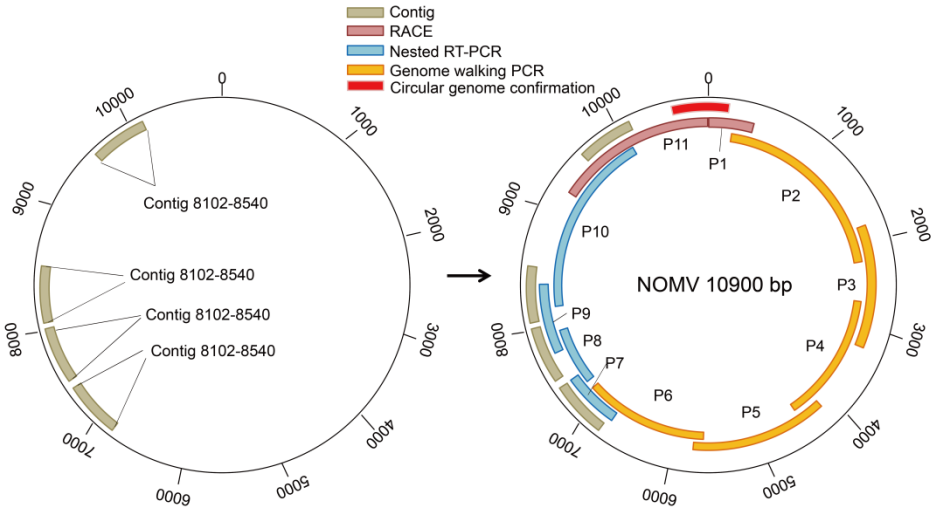
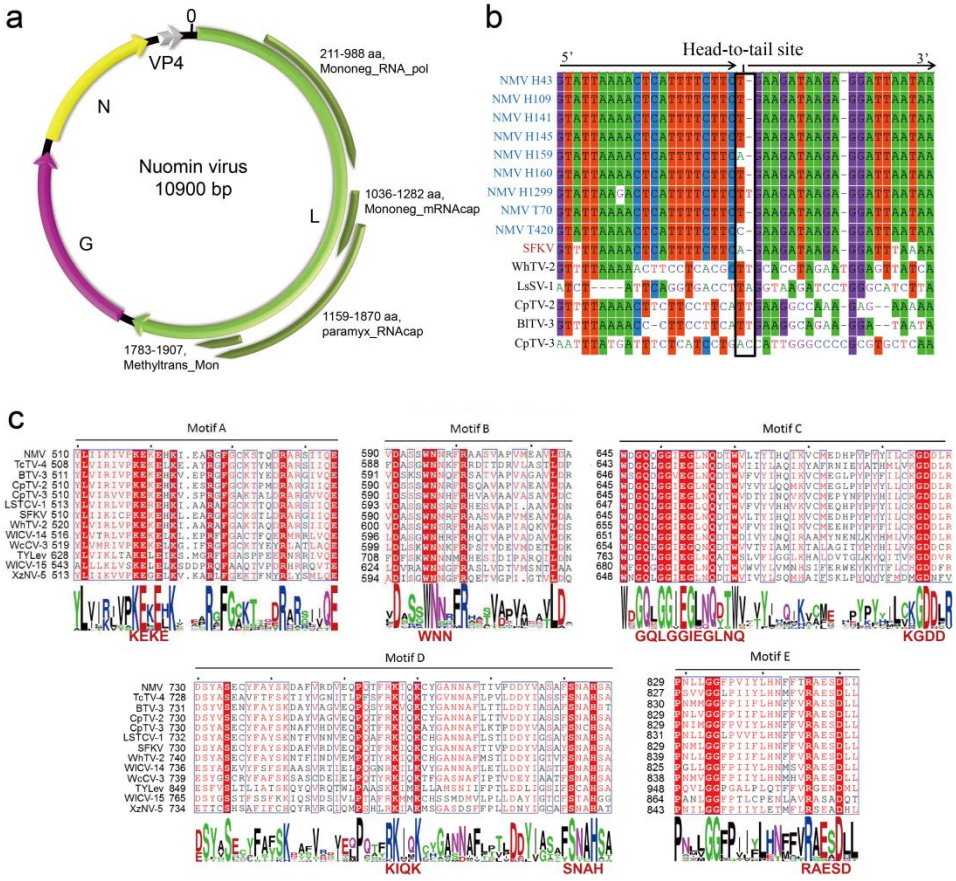
Extended Data



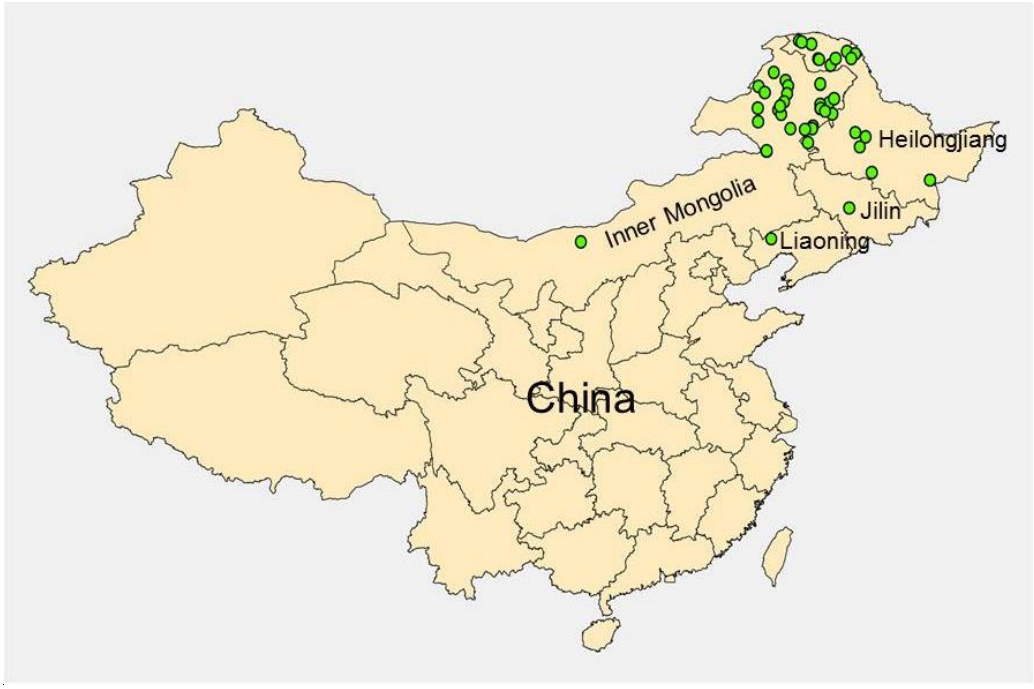
Extended Data Fig. 1. Development of real-time RT-PCR for NOMV detection. **a,** The standard curve of the real-time RT-PCR using the primers in Supplemental Table S1. **b**, The melting curve of real-time RT-PCR. **c**, Amplification plot of plasmids containing the NOMV N gene serially diluted from 10^8 to 10^1 copies/μL by real-time RT-PCR, and the detection limitation was about 10 copies/μL. **d**, Amplification products of real-time RT-PCR analyzed by agarose gel.



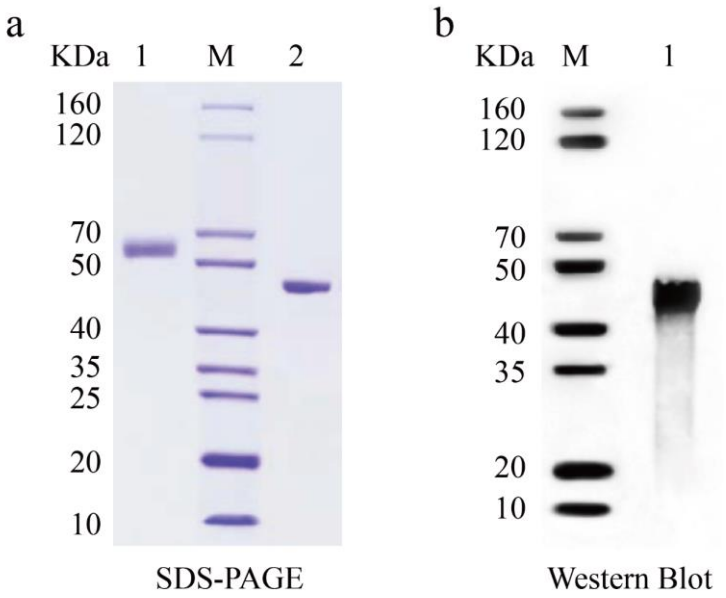
Extended Data Fig. 2. Strategy of sequencing the genome of NOMV. The sequence contigs (brown boxes) generated by metagenomic analysis are marked with the mapped positions in the genome of Suffolk virus (GenBank accession no. KM460042, Supplemental Table S1). The complete genome was obtained using nested RT-PCR (blue boxes) with overlapped primers and genome walking (orange boxes), or rapid amplification of complementary DNA ends (RACE) (dark red boxes). The circular genome form was further confirmed by nested RT-PCR that can amplify the sequence that cover the head-to-tail site (red boxes). The primers used in this study are listed in Supplemental Table 6.



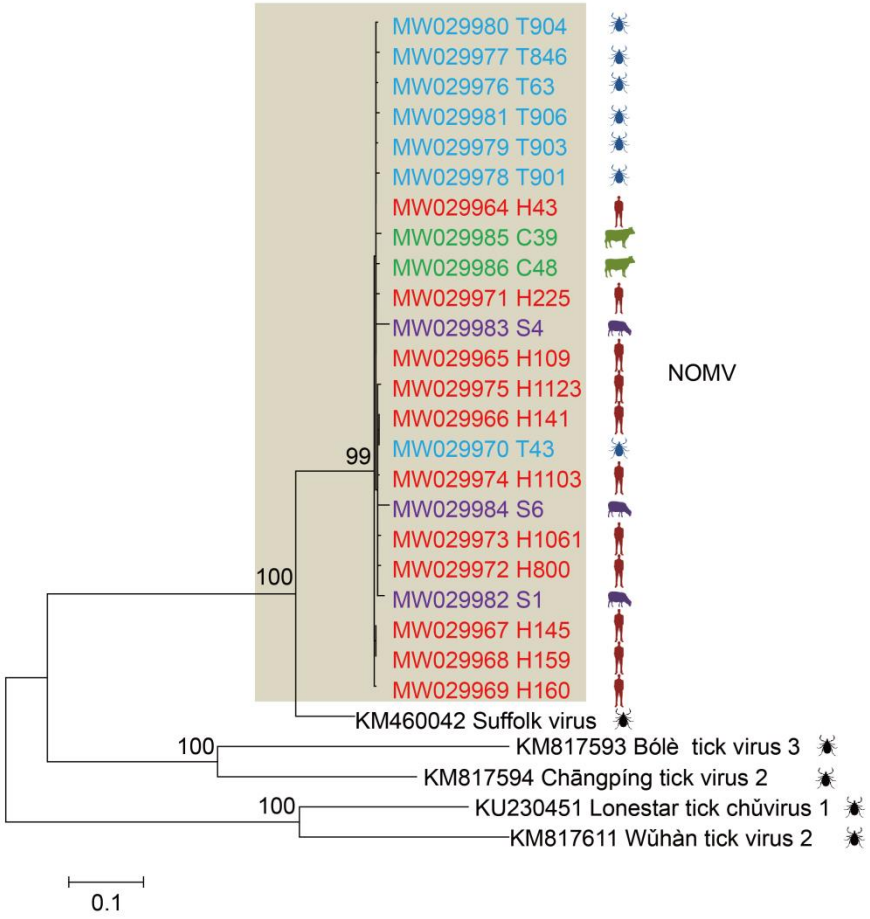
Extended Data Fig. 3. Molecular characterization of NOMV. a, Schematic genome organization of NOMV. The predicted open reading frames (ORFs) and their super families are shown in boxes. b, Comparison of the head-to-tail sequences of circular chuviruses. c, The conserved motifs in RNA dependent RNA polymerase in NOMV and other chuviruses. Mononeg_RNA_pol, Mononegavirales RNA dependent RNA polymerase; Mononeg_mRNAcap, Mononegavirales mRNA-capping region V; paramyx_RNAcap, mRNA capping enzyme in the Paramyxovirus family; Methyltrans_Mon, Virus-capping methyltransferase; TcTV-4, Tǎchéng tick virus 4; BTV-3, Bólè tick virus 3; WlCV-14, Wēnlǐng crustacean virus 14; CpTV-2, Chāngpíng tick virus 2; WcCV-3, Wǔchāng cockroach virus 3; CpTV-3, Chāngpíng tick virus 3; LSTCV-1, Lonestar tick chǔvirus 1; SFKV, Suffolk virus; TYLeV, Tàiyuán leafhopper virus; WlCV-15, Wēnlǐng crustacean virus 15; WhTV-2, WhTV- tick virus 2; XzNV-5, Xīnzhōu nematode virus 5.



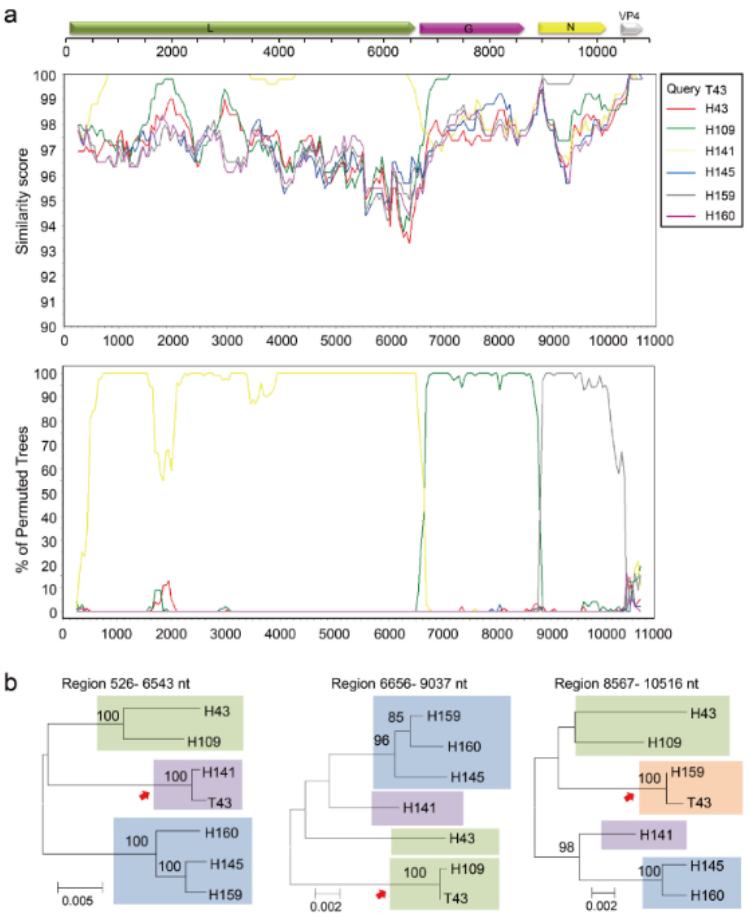
Extended Data Fig. 4. Geographic distribution of patients with NOMV infection in China. Green circles indicate the locations of the patients with confirmed NOMV infection.



Extended Data Fig. 5. Expressed recombinant nucleoprotein (N) of NOMV. a, Sodium dodecyl sulphate-polyacrylamide gel electrophoresisof purified his-tagged nucleoprotein of NOMV. Lane 1, Bovine serum albumin; Lane 2, Purified his-tagged nucleoprotein of NOMV; Lane M, Protein molecular weight marker. **b**, Western-blot analysis of recombinant nucleoprotein using anti-His monoclonal antibody. Lane 1, Purified his-tagged nucleoprotein of NOMV; Lane M, Protein molecular weight marker.



Extended Data Fig. 6. Phylogenetic analysis of the partial RdRp gene of NOMVs. The phylogenetic trees were generated by MEGA 5 software and analyzed by the Maximum likelihood method with the Jukes-Cantor model. Bootstrap testing of 1000 replicates was performed, and the bootstrap values are indicated. Sequences are identified by their GenBank accession numbers, followed by the virus name. The scale bars indicate 0.1 substitutions per site. Bootstrap values higher than 70% are shown. Four hosts of NOMVs with human, tick, sheep, and cattle are shaded red, blue, purple, and green, respectively. The silhouette figures represent the virus hosts.



Extended Data Fig. 7. Recombination instances appeared among NOMV strains. **a**, Similarity plot and bootscanning analysis of the T43 (tick source) strain with other human source NOMV strains. The analysis was conducted via Simplot v3.5.1 using a sliding window of 500 nucleotides moving in steps of 50 nucleotides. The genome of Nuomin virus strains T43 (MW029970) serve as query sequences. Full-length genome sequence of NOMV strains from six tick-bite patients (H43, MW029964; H109, MW029965; H141, MW029966; H145, MW029967; H159, MW029968; H160, MW029969) are included for the analysis. Several possible recombination events are suggested. **b**, ML phylogenetic trees of the three breakpoints from different NOMV isolates. The trees are midpoint-rooted and horizontal branches are drawn to a scale of nucleotide substitutions per site. Major clade/lineages are shaded with blue, green, purple, and orange, respectively. Phylogenetic incongruence, suggestive of recombinant, is indicated with colored arrowheads.

Extended Data Table 1. Detection method of tick-borne pathogens in this study

Pathogen*	Detection method	Target gene	Reference
TBEV	Nested RT-PCR	NS1	Achazi et al[1]
SFTSV	Real-time RT-PCR	S gene	Liu et al[2]
ALSV	Real-time RT-PCR	NS3	Wang et al[3]
Borrelia burgdorferi sensu lato	Nested PCR	ITS, 16S rRNA	Bonczek et al[4]
Borrelia miyamotoi	Real-time PCR	16S rRNA	Jiang et al[5]
Anaplasma spp.	PCR	16S rRNA, groEL	Wei et al[6]
Babesia spp.	Nested PCR	18S rRNA	Wei et al[6]
Rickettsia spp.	PCR	16S rRNA	Liu et al[7]

*TBEV, tick-borne encephalitis virus; SFTSV, severe fever with thrombocytopenia syndrome; ALSV, Alongshan virus

Extended Data Table 2. Similarity (%) between NOMVs and other chuviruses*

Chuvirus	L (%)		G (%)		N (%)	
	aa	nt	aa	nt	aa	nt
NOMV	98.9–100.0	97.8–99.7	99.5–99.9	98.8–100.0	99.8–100.0	99.3–100.0
Circular	45.4–83.4	55.1–74.9	68.8–89.6	54.0–60.6	20.6–93.5	69.5–83.6
Bi-circular	45.4–54.1	53.4–59.1	69.7–72.2	53.3–64.2	20.4–90.3	68.9–83.6
Unsegmented	17.1–47.0	43.3–54.7	36.4–73.6	7.7–60.8	20.1–89.1	38.5–73.0
Bi-segmented	48.1–48.6	54–56.1	67.8–72.2	53.1–62.3	87.0–89.4	68.6–74.1

*Chuviruses other than NOMV are listed in Supplemental Table S4. L, the large polymerase protein; G, glycoprotein; N, nucleoprotein.

Extended Data Table 3. Demographic information on the studied populations in this study

Epidemiology character	Patients with NOMV (n=54)	Hospitalized patients (n=830)	Control population (n=200)
	no. (%)		
Region			
Inner Mongolia	34 (63.0)	586 (70.6)	140 (70)
Heilongjiang	18 (33.4)	228 (27.5)	60 (30)
Jilin	1 (1.8)	12 (1.4)	0
Liaoning	1 (1.8)	4 (0.5)	0
Year			
2017	14 (26.0)	348 (41.9)	100 (50)
2018	20 (37.0)	310 (37.3)	100 (50)
2019	20 (37.0)	172 (20.8)	
Age			
<40	16 (29.6)	198 (23.9)	40 (20)
40-60	34 (63.0)	556 (67.0)	140 (70)
>60	4 (7.4)	76 (9.1)	20 (10)
Sex			
Male	36 (66.7)	573 (69.0)	140 (70)
Female	18 (33.3)	257 (31.0)	60 (30)
Month distribution			
Before May	4 (7.4)	78 (9.4)	0
May to July	45 (83.3)	682 (82.2)	200 (100)
After July	5 (9.3)	70 (8.4)	0
Profession			
Field worker*	48 (88.9)	798 (96.1)	0
others	6 (11.1)	32 (3.9)	200 (100)
Tick bite history			
Yes	54 (100)	819 (98.7)	0
No	0	0	200 (100)
nd	0	11 (1.3)	0

*Field worker include farmer, forestry worker, or other people who living in wooded and hilly areas and working in fields.

Extended Data Table 4. Serological and molecular detection of NOMV-infected patients in China*

Patient No.	Sex	Age (year)	Province	Sampling time	ELISA†		MNT‡
					IgM	IgG	
H125 (AP)	F	49	Inner Mongolia	9	160	10	<10
H125 (CP)	F	49	Inner Mongolia	30	<10	80	40
H150 (AP)	M	54	Inner Mongolia	5	10	<10	<10
H150 (CP)	M	54	Inner Mongolia	25	<10	80	40
H196	M	29	Inner Mongolia	14	10	40	40
H239	M	51	Inner Mongolia	13	80	10	<10
H252	M	49	Heilongjiang	14	40	10	10
H410	F	35	Inner Mongolia	19	10	80	40
H418 (AP)	M	54	Inner Mongolia	6	10	<10	<10
H418 (CP)	M	54	Inner Mongolia	37	<10	80	40
H639 (AP)	M	56	Inner Mongolia	9	40	<10	<10
H639 (CP)	M	56	Inner Mongolia	22	<10	160	80
H664	F	39	Inner Mongolia	15	20	40	40
H1171	F	52	Inner Mongolia	13	80	10	<10
H1261	F	56	Heilongjiang	15	10	20	10
H1299 (AP)	M	25	Inner Mongolia	7	10	10	<10
H1299 (CP)	M	25	Inner Mongolia	26	<10	80	80
H1300	F	69	Inner Mongolia	17	10	<10	<10
H1433 (AP)	M	51	Inner Mongolia	10	80	<10	<10
H1433 (CP)	M	51	Inner Mongolia	31	<10	160	80

*All patients had NOMV infection as confirmed by reverse-transcriptase–polymerase-chain-reaction assay. Sampling time indicate patients' serum samples collection days after tick bite; AP denotes acute phase, and CP convalescent phase.

†Enzyme-linked immunosorbent assay (ELISA) end-point titers are reported as the reciprocal of the highest dilution of serum that showed a mean OD value higher than the cut-off line.

‡Microneutralization (MNT) end-point titers are reported as the reciprocal of the highest dilution of serum that could inhibit viral infection.

Extended Data Table 5. Clinical characteristic of patients with NOMV infection

Clinical	Patients with Symptoms (%)
Headache	43 (79.6)
Fever	43 (79.6)
Depression	31 (57.4)
Dizziness	26 (48.1)
Fatigue	21 (38.8)
Myalgia or	21 (38.8)
Poor appetite	18 (33.3)
Nausea	16 (29.6)
Cough	11 (20.3)
Chest pain	11 (20.3)
Rash or petechiae	9 (16.6)
Shortness of	9 (16.6)
Vomit	6 (11.1)
Diarrhea	3 (5.5)
Lymphadenopathy	3 (5.5)
Palpitation	2 (3.7)
Abdominal pain	2 (3.7)
Chill	2 (3.7)

Extended Data Table 6. Laboratory findings in patients with NOMV infection

Results (no. of patients)	Normal	Increased	Decreased
	No. of patients (%)		
Blood routine (n=53)			
Leucocyte (× 10^9 per L)	35 (66.0)	11 (20.8)	7 (13.2)
Neutrophil (× 10^9 per L)	37 (69.8)	13 (24.5)	3 (5.7)
Lymphocyte (× 10^9 per L)	38 (71.7)	0 (0)	15 (28.3)
Monocyte (× 10^9 per L)	35 (66)	16 (30.2)	2 (3.8)
Hemoglobin (g/L)	38 (71.7)	2 (3.8)	13 (24.5)
Platelet (× 10^9 per L)	41 (77.4)	4 (7.5)	8 (15.1)
Coagulation function			
Prothrombin time (s, n=50)	47 (94)	3 (6.0)	0 (0)
Activated partial thromboplastin time (s, n=48)	43 (89.6)	4 (8.3)	1 (2.1)
Fibrinogen (g/L, n=48)	41 (85.4)	6 (12.5)	1 (2.1)
D-dimer (mg/L, n=5)	2 (40.0)	3 (60.0)	0 (0)
Blood biochemistry			
Albumin (g/L; n=17)	8 (47.1)	0 (0)	9 (52.9)
Alanine aminotransferase (U/L; n=52)	35 (67.3)	17 (32.7)	0 (0)
Aspartate aminotransferase (U/L; n=52)	27 (51.9)	23 (44.2)	2 (3.9)
Total bilirubin (umol/L; n=44)	34 (77.3)	10 (22.7)	0 (0)
Lactate dehydrogenase (U/L; n=19)	14 (73.7)	5 (26.3)	0 (0)
Myoglobin (ng/ml; n=17)	6 (35.3)	1 (5.9)	10 (58.8)
L-γ-glutamine (U/L; n=50)	37 (74.0)	13 (26.0)	0 (0)
Cholinesterase (U/L; n=44)	37 (84.1)	0 (0)	7 (15.9)
Serum creatinine (μmol/L; n=53)	48 (90.6)	5 (9.4)	0 (0)
Creatine kinase (μmol/L; n=20)	18 (90)	2 (10)	0 (0)
Uric acid (umol/L; n=54)	37 (68.5)	10 (18.5)	7 (13.0)
Apolipoprotein AI (g/L; n=29)	7 (24.1)	0 (0)	22 (75.9)
Direct bilirubin (umol/L; n=44)	30 (68.2)	14 (31.8)	0 (0)
Indirect bilirubin (umol/L; n=44)	35 (79.5)	9 (20.5)	0 (0)
Bile acid (umol/L; n=44)	37 (84.1)	7 (15.9)	0 (0)
Triglyceride (mmol/L; n=48)	32 (66.7)	16 (33.3)	0 (0)
High-density lipoprotein (mmol/L; n=48)	31 (64.6)	0 (0)	17 (35.4)
Homocysteine (umol/L; n=16)	0 (0)	16 (100)	0 (0)
Urea (mmol/L; n=54)	37 (68.5)	10 (18.5)	7 (13.0)
Total carbon dioxide (mmol/L; n=54)	35 (64.8)	1 (1.9)	18 (33.3)
Sodium (mmol/L; n=52)	41 (78.8)	0 (0)	11 (21.2)
Glucose (mmol/L; n=52)	24 (46.2)	28 (53.8)	0 (0)
C-reactive protein (mg/L; n=33)	11 (33.3)	22 (66.7)	0 (0)

Extended Data Table 7. Detection of NOMV RNA in ticks by nested RT-PCR[*]

Tick species	Heilongjiang		Inner Mongolia[†]		Jilin[†]		Total	
	No. positive pools/No. tested pools (No. ticks in positive pools/ No. ticks tested)	Prevalence (95% CI)	No. positive pools/No. tested pools (No. ticks in positive pools/ No. ticks tested)	Prevalence (95% CI)	No. positive pools/No. tested pools (No. ticks in positive pools/ No. ticks tested)	Prevalence (95% CI)	No. positive pools/No. tested pools (No. ticks in positive pools/ No. ticks tested)	Prevalence (95% CI)
Ixodes persulcatus	17/19 (170/284)	12.8 (7.7-23.2)	5/11 (75/165)	3.7 (1.4-8.5)	nd	nd	22/30 (245/449)	8.2 (5.3-12.6)
Ixodes crenulatus	17/21 (255/314)	10.0 (6.1-16.8)	3/7 (45/105)	3.3 (0.9-9.5)	nd	nd	20/28 (300/419)	7.8 (4.9-12.2)
Haemaphysalis concinna	1/2 (10/29)	3.2 (0.2-21.7)	nd	nd	3/9 (45/135)	2.5 (0.7-6.9)	4/11 (55/164)	2.8 (1.0-7.0)
Haemaphysalis Iongicornis	0/3 (0/45)	0	1/6 (15/20)	5.8 (0.4-42.7)	18/70 (270/1050)	2.0 (1.2-3.0)	19/79 (285/1115)	1.9 (1.2-3.0)
Total	35/45 (435/672)	9.4 (6.6-13.3)	9/24 (135/290)	4.0 (2.0-7.4)	21/79 (315/1185)	2.0 (1.3-3.0)	65/148 (885/2147)	3.9 (3.1-5.0)

[*]The prevalence was expressed as the minimum infection rate that was calculated by maximum likelihood estimation (MLE) using the program PooledInfRate.

[†]nd, no data.

Extended Data Table 8. Nucleotide sequences of NOMVs obtained in this study

Sequence_ID	GenBank accession no.	Strain	Gene*	Sampling sites	Hosts
Seq1	MW029964	H43	Complete genome	Yakeshi, Inner Mongolia	Homo sapiens
Seq2	MW029965	H109	Complete genome	Alongshan, Inner Mongolia	Homo sapiens
Seq3	MW029966	H141	Complete genome	Mohe, Heilongjiang	Homo sapiens
Seq4	MW029967	H145	Complete genome	Oroqen, Inner Mongolia	Homo sapiens
Seq5	MW029968	H159	Complete genome	Yakeshi, Inner Mongolia	Homo sapiens
Seq6	MW029969	H160	Complete genome	Tahe, Heilongjiang	Homo sapiens
Seq7	MW029971	H225	Partial RdRp	Moqi, Inner Mongolia	Homo sapiens
Seq8	MW029972	H800	Partial RdRp	Moqi, Inner Mongolia	Homo sapiens
Seq9	MW029973	H1061	Partial RdRp	Mohe, Heilongjiang	Homo sapiens
Seq10	MW029974	H1103	Partial RdRp	Mohe, Heilongjiang	Homo sapiens
Seq11	MW029975	H1123	Partial RdRp	Tahe, Heilongjiang	Homo sapiens
Seq12	MW029970	T43	Complete genome	Alongshan, Inner Mongolia	*Ixodex persulcatus*
Seq13	MW029976	T63	Partial RdRp	Alongshan, Inner Mongolia	*Ixodex persulcatus*
Seq14	MW029977	T846	Partial RdRp	Yichun, Heilongjiang	*Ixodex persulcatus*
Seq15	MW029978	T901	Partial RdRp	Yichun, Heilongjiang	*Ixodes crenulaus*
Seq16	MW029979	T903	Partial RdRp	Yichun, Heilongjiang	*Ixodes crenulaus*
Seq17	MW029980	T904	Partial RdRp	Yichun, Heilongjiang	*Ixodes crenulaus*
Seq18	MW029981	T906	Partial RdRp	Yichun, Heilongjiang	*Ixodes crenulaus*
Seq19	MW029982	S1	Partial RdRp	Oroqen, Inner Mongolia	Sheep
Seq20	MW029983	S4	Partial RdRp	Oroqen, Inner Mongolia	Sheep
Seq21	MW029984	S6	Partial RdRp	Oroqen, Inner Mongolia	Sheep
Seq22	MW029985	C39	Partial RdRp	Genhe, Inner Mongolia	Cattle
Seq23	MW029986	C48	Partial RdRp	Genhe, Inner Mongolia	Cattle

*RdRp, RNA dependent RNA polymerase;

Extended Data Table 9. Recombination events of NOMVs detected using the RDP package*

Strain	Major parent	Minor parent	Breakpoint positions (bp, 95% CI)		RDPRCS	Tools (Methods with significant *P*-value for this recombination)
			Begin	End		
H141	T43	Unknown	526 (221-621)	6543 (6425-9092)	0.525	RDP, GENECONV, MaxChi, Chimaera, Siscan, 3Seq, BootScan
H109	T43	H43	6656 (6449-6959)	9037 (8392-9092)	0.499	RDP, GENECONV, MaxChi, Chimaera, Siscan, 3Seq, BootScan
H159	T43	H145	8567 (8494-9050)	10516 (10105-141)	0.719	RDP, GENECONV, MaxChi, Chimaera, Siscan, 3Seq, BootScan

*Minor parent, Parent contributing the smaller fraction of sequence; Major parent, Parent contributing the larger fraction of sequence; The sequence listed as unknown was used to infer the existance of a missing parental sequence; RDPRCS, The RDP recombination consensus score.

Reference：

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