Identification of a new chuvirus associated with febrile illness in China

Liu Quan (liuquan1973@hotmail.com)
Jilin University https://orcid.org/0000-0003-3411-3196

Ze-Dong Wang
Jilin University, First Hospital

Yuan Gao
College of Veterinary Medicine, Northeast Agricultural University

Xiaolong Lv
Inner Mongolia General Forestry Hospital

Shuzheng Han
Inner Mongolia General Forestry Hospital

Xu Zhang
Foshan University https://orcid.org/0000-0002-1628-9895

Jian-Wei Shao
School of Life Sciences and Engineering, Foshan University

Chen Chen
The First Hospital of Jilin University

Liang Li
Military Veterinary Institute, Academy of Military Medical Sciences

Zhi-Jun Hou
College of Wildlife and Protected Area, Northeast Forestry University

Liyan Sui
Department of Emerging Infectious Diseases, The First Hospital of Jilin University

Yinghua Zhao
The First Hospital of Jilin University

Bo Wang
Hainan General Hospital

Wei Wang
Inner Mongolia General Forestry Hospital https://orcid.org/0000-0003-1221-6004

Mingxin Song
College of Veterinary Medicine, Northeast Agricultural University

Letter

Keywords: chuvirus, Nuomin virus, human febrile illness, ticks

Posted Date: December 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-104938/v1

Abstract

Chuviruses belong to the genus *Mivirus* in the newly classified family *Chuviridae*, and their medical significance remains undetermined[1,2]. Here, we identified a novel chuvirus, Nuomin virus (NOMV), from febrile patients in China. NOMV had a circular genome and presented enveloped spherical particles with a diameter of approximately 120–150 nm. Phylogenetically, NOMV formed a separate clade in the *Chuviridae* family, with 17.1–83.4% sequence identity to other chuviruses. NOMV infection was confirmed in 54 patients who had a tick-bitten history during 2017–2019, while both virus-specific antibodies and RNA were tested negative in healthy cohorts. The typical symptoms are characterized by fever and headache. Seroconversion or a four-fold elevation of antibody titers was observed in the available specimens of convalescent phase. NOMV was also detected in hard ticks, sheep, and cattle in northeastern China. Our findings showed that a novel chuvirus is associated with human febrile illness in China, necessitating investigation of this emerging virus in the tick-endemic areas worldwide.

Main Text

Chuviruses, a group of single-stranded negative sense RNA viruses, have been discovered in arthropods, snakes, fishes, and nematodes, with the unsegmented, bi-segmented, circular, and bi-circular genomic structures that encodes the large protein (L), glycoprotein (G), nucleoprotein (N), and an uncharacterized viral protein (VP)[3–7]. Chuviruses are genetically situated between segmented and unsegmented negative-sense RNA viruses[4]. Most chuviruses were discovered in the middle and lower reaches of the Yangzi River region, which was called Chu in ancient China[6,8]. Chuviruses have also been found in United States of America[3], Brazil[9], Australia[10], and other countries[11–13]. However, their relevance to public health remains unclear. Here, we identified a new chuvirus, Nuomin virus (NOMV), that is associated with human febrile illness in China.

In May 2017, a 29-year-old male farmer in the Nuomin town in Inner Mongolia, China was admitted to the local hospital, due to the clinical symptoms of fever, headache, nausea, and vomiting. The patient had a tick bite before illness onset. He was infected with tick-borne encephalitis virus (TBEV) in 2014, and received inactivated TBEV vaccination in 2017. TBEV-specific IgG antibodies were detected positive, but viral RNA was negative in the serum sample. Other tick-borne pathogens, including Alongshan virus, severe fever with thrombocytopenia syndrome virus (SFTSV), *Babesia* spp., *Anaplasma* spp., *Rickettsia* spp., Lyme disease spirochetes, were tested negative (Extended Data Table 1)[14–20]. To hunt the potential causative agent, the blood sample was used for metagenomic analysis, which resulted to several contigs annotated to Suffolk virus (SFKV, Supplemental Table S1)[3].

Despite presence of numerous genomic sequences of new chuviruses in the last five years, none has been isolated. We isolated the virus from the patient's blood sample by using Vero and BHK-21 cells, but no clear cytopathic effect was observed. Purified virions showed enveloped spherical particles, with a diameter of 120–150 nm under electronic microscopy (Fig. 1a). Viral particles in ultrathin sections could

be observed in the cytoplasmic vacuoles of infected BHK-21 cells (Fig. 1b). We designated Nuomin virus (NOMV), and the disease was called Nuomin fever.

Real-time RT-PCR assay were established for quantitative detection of the NOMV (Extended Data Fig. 1) and evaluated the viral infectivity of multiple cell lines, including Vero, BHK-21, hepatocellular carcinoma cell (SMMC-7721), amniotic cell (WISH), and epithelial colorectal adenocarcinoma cell (Caco-2). The results showed that NOMV replicates more efficiently in BHK-21 and SMMC-7721 cells than in SMMC-7721 and WISH cells (Fig. 2). We later isolated five additional strains from inpatients and one strain from *Ixodes persulcatus*. This was the first isolation of virus species in the family *Chuviridae*.

The complete genome of NOMV strains was obtained based on metagenomic analysis (Extended Data Fig. 2). The circular genome structure of NOMV was confirmed with around-the-genome RT-PCR. NOMV genome sequence shared high similarity with SFKV as compared with other chuviruses (Extended Data Fig. 3). Interestingly, the head-to-tail sequences of NOMVs had a T-to-A/C substitution and an insertion of T. The length of NOMV genome was 10,900 bp, and contained 4 open reading frames (ORFs) encoding the large protein (L, 6516 nt), glycoprotein (G, 2001 nt), nucleoprotein (N, 1278 nt), and an unknown viral protein (VP4, 324 nt), respectively. ORF1 encoded a 2171-aa large protein (L), which had 17.1–83.4% identity to other chuviruses (Extended data Table 2), and characterized by the conserved domains responsible for viral RNA replication (Extended Data Fig. 3)[6]. ORF2 encoded a putative 666-aa glycoprotein (G), with 36.4–89.6% similarity to other chuviruses. ORF3 encoded a putative 425-aa nucleoprotein (N), with 20.1–93.5% identity to other chuviruses. ORF4 encoded a 107-aa protein (VP4), which shared 54.4% sequence identity to Suffolk virus (SFKV). Due to a lack of homology to proteins outside of the family *Chuviridae*, the potential function of VP4 remains unknown.

To determine the evolutionary relationships between NOMV and other chuviruses, phylogenetic trees were constructed with amino acid sequences of the L, G, and N proteins, respectively. NOMVs formed a separate clade from the viruses in the *Chuviridae* family and were clustered with viral members that have a circular genome; circular, bi-circular, or bi-segmented chuviruses may evolve from unsegmented viruses (Fig. 3). Notably, almost all chuviruses that contain a circular genome has been discovered in ticks (Fig. 3).

During 2017–2019, a total of 54 patients were confirmed NOMV infection by real-time RT-PCR. Of these patients, 14 were found in 2017, 20 in both 2018 and 2019; 34 were from Inner Mongolia, 18 were from Heilongjiang, and one each from Jilin and Liaoning (Extended Data Fig. 4); 66.7% (36/54) of patients were men and their ages ranged from 22 to 69 years old, with 63.0% (34/54) between 40 and 60 years old; 48 (88.9%) were field workers, and all had clear history of tick bites before the onset of illness; 83.3% of patients occurred during May to July (Extended Data Table 3).

The median time from tick bite to illness onset (incubation period, IP) was 5 days (IQR, 2–9 days), from tick bite to hospital admission was 8 days (IQR, 6–14 days), and from tick bite to discharge was 20 days (IQR, 17–27 days).

We tested the serologic responses against NOMV by ELISA (Extended data Fig. 5), showing that all serum samples were IgM-positive in available serum samples from 14 patients at the acute period (AP), and IgG antibodies were gradually increased; 78.6% (11/14) of patients had a low level of neutralizing antibody at the AP, while high levels of neutralizing antibody were found in the CP specimens (Extended Data Table 4). Seroconversion or at least 4 times as high as the titer in the AP specimens were detected in the six serum samples at the CP (Fig. 4).

NOMV caused non-specific manifestations including fever and headache. Other clinical findings may include depression, dizziness, fatigue, myalgia, arthralgia, nausea, cough, rash, or petechiae (Extended Data Table 5). Biochemical analysis of blood showed elevated monocytes (30.2%), neutrophils (24.5%), and decreased lymphocytes (28.3%). The most common abnormal biochemical indicators included elevated high-sensitivity C-reactive protein (66.7%) and decreased apolipoprotein AI (75.9%). Liver injury was shown in approximately 30% of patients, and myocardial injury in approximately 10%, as indicated by increased serum aspartate aminotransferase (44.2%), alanine aminotransferase (32.7%), lactate dehydrogenase (26.3%), and creatine kinase (10%) (Extended Data Table 6).

Patients were given a combination of ribavirin and benzylpenicillin sodium. Ribavirin was given intravenously 0.5 g per day, and benzylpenicillin sodium was injected intramuscularly 4 million units per day. The clinical symptoms often resolved after treatment for 7 to 14 days.

We collected 2,147 hard ticks in the hilly and wooded regions where NOMV-infected patients were usually bitten. These ticks were divided into 148 pools and NOMV was detected by nested RT-PCR (Supplementary Table S5), showing a prevalence of 3.9%, i.e., 8.2% in *Ixodes persulcatus*, 7.8% in *Ixodes crenulatus*, 2.8% in *Haemaphysalis conicinna*, and 1.9% in *Haemaphysalis longicornis*. A higher prevalence was found in ticks in Heilongjiang (9.4%) than that in Inner Mongolia (4.0%) and Jilin (2.0%) provinces ($p < 0.5$) (Extended Data Table 7). We also detected viral RNA in the serum specimens of sheep and cattle in Inner Mongolia by real-time RT-PCR, revealing a prevalence of 21.7% (40/184) in sheep and 31.4% (79/252) in cattle. Partial RdRp genes of NOMV from ticks, cattle and sheep were obtained by using nested RT-PCR (Supplemental Table S5), and phylogenetic analysis showed that they were closely related to the human strains (Extended Data Fig. 6 and Table 8).

To explore the evolutionary origin of NOMVs, potential recombination was analyzed with the Recombination Detection Program 4.0 using available genomes. Several breakpoints were detected, which were also supported by similarity plot and bootscan analysis (Extended Data Fig. 7 and Table 9). Breakpoints were located at the nucleotides 526 and 6543 in L gene of H141, and at the nucleotides 6,656 and 9,037 in the G and partial N genes of H109, thus generating recombination fragments covering nucleotides 8567–10516 including partial G, N, and partial VP4 gene. Phylogenetic analysis suggested that human virus strains H141, H109, and H159 were the potential descendent of T43 detected in ticks.

The present study identified a new chuvirus in the family *Chuviridae*, the likely agent responsible for a febrile illness in China. Viral RNA positive in all patients, and viral protein seroconversion or at least 4 times as high as the titer observed in the available specimens at CP provides evidence of an association

between the new chuvirus and the febrile illness. However, there are still many questions to be answered. Firstly, the association between the new chuvirus and the disease has not been confirmed by animal infection experiments to fulfil the Koch's postulates. Secondly, though the virus has been detected in several tick species, their vectorial capacity needs to be further confirmed. Thirdly, it appears that NOMV cannot be transmitted from person to person, however, the virus should be closely monitored whether to evolve into a more virulent one. Fourthly, the pathogenesis mechanism should be elucidated and antiviral drugs and vaccines be developed for this emerging infectious disease. Lastly, the differential diagnosis of tick-borne diseases should include this emerging virus, whose public health significance makes it necessary to further investigate in the tick-endemic areas worldwide.

Methods

Study design and sample collection

We recruited patients who reported being bitten by ticks in the local hospital of Inner Mongolia in China during 2017–2019, and the blood specimens were collected to investigate potential tick-borne pathogens. A standardized questionnaire was used to collect data on tick exposure, demography, and medical history of each patient. The clinical symptoms and laboratory tests were obtained from the medical records.

We included the patients who were detected positive for viral RNA in the blood sample by real-time RT-PCR, and excluded the patients who had co-infection or infected with other pathogens. Two hundred healthy people in the same areas were included as controls.

The sheep and cattle blood samples were collected from Hulunbuir, Inner Mongolia in 2017. Serum was separated and stored at -80°C until use. Ticks were collected by flagging vegetation in northeastern China from May, 2015 to July, 2016, and classified to species by the morphological traits and molecular identifications[20,21].

Viral metagenomics

Viral metagenomics was conducted as described elsewhere [8]. Briefly, RNA was extracted from blood sample and reverse transcribed into double strand cDNA using the random primer 5´-GCCGGAGCTCTGCAGATATCNNNNNN-3´and a Klenow fragment. Sequence-independent single-primer amplification was used to amplify the double strand cDNA in a 50 μL reaction system that contained 25 μL Q5 high-fidelity Mix (NEB), 3 μL of the above double strand cDNA mixture, 3 μL primer (5´-GCCGGAGCTCTGCAGATATC-3´) and 19 μL ddH_2O. The PCR products were purified for library construction and high-throughput sequencing in the Beijing Genome Institute (BGI, Shenzhen, China).

RNA extraction, nested RT-PCR, and real-time RT-PCR

Nested RT-PCR and real-time RT-PCR assays were set up with the aim to detect virus in serum samples and other samples. Total RNA was extracted and cDNA was synthesized by PrimeScript™ RT reagent Kit

(TaKaRa). The primers based on the L gene of NOMV were designed according to the viral metagenomic results and listed in Supplemental Table S5. For nested RT-PCR, the 25-μL PCR reaction mixture containing 12.5 μL of Premix Taq (TaKaRa), 1 μL of each primer, 1μL of template cDNA and 9.5 μL ddH_2O. Amplification parameters were 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 50°C for 30 s, 72°C for 20-30 s, and a final extension at 72°C for 10 min. For real-time PCR, the 20-μL qPCR reaction mixture contained 10 μL 2×SYBR premix Ex TaqII (Takara), 0.4 μM of each primer and 1 μL template cDNA. Amplification parameters were 95°C for 30 s followed by 50 cycles at 95 °C for 5 s, 60 °C for 30 s, and 95 °C for 15 s, 60 °C for 30 s, 95 °C for 15 s for melt curve stage in an Eco^{TM} Real-Time PCR System.

Genetic analysis

The complete genome of NOMV was obtained through nested RT-PCR with overlapping primers designed according to the metagenomic sequences as described in Extended data Fig. 2. The circular genomic form of NOMV was confirmed by around-the-genome nested RT-PCR[8]. Partial RdRp gene of NOMV from different hosts used for phylogenetic analysis were obtained by nested RT-PCR. All the primers used were listed in Supplemental Table S5-S6. PCR products were sequenced using the standard methods. Phylogenetic analyses were performed with the maximum likelihood method in the Molecular Evolutionary Genetics Analysis 5.0 (MEGA 5.0). The RdRp gene of typical viruses in the order *Mononegavirales* (132 strains) and 57 chuviruses were all used for phylogenetic analysis in the study (Supplemental Table S2-S3).

Virus isolation

BHK-21 and Vero cells were used to isolate the new virus. They were grown in Dulbecco's modified eagle medium supplemented with 2% fetal bovine serum, 1000 units/ml penicillin and 1 mg/ml streptomycin antibiotics. The blood sample from the index patient was centrifuged at 12,000 g for 10 min; the supernatant was diluted 10 times and inoculated onto a confluent monolayer of BHK-21 or Vero cells for 1.5 h and replaced with DMEM supplemented with 1% FBS. The cells were cultured at 37°C in 5% CO_2 and monitored daily for cytopathic effect (CPE). Three blind passages, with a four-day interval between every passage, were conducted for each sample. The culture supernatant and cellular pellet of each passage were tested for the virus by real-time RT-PCR. Electron microscopy analysis was conducted as described previously[22]. Virus was quantitated by fluorescent focus assay and viral titers were determined as fluorescent focus units (FFU) per mL[23].

Recombination analysis

Full-length genomic sequence of NOMV tick strain T43 (MW029970) was aligned with the available NMV strains from patients (H43, MW029971; H109, MW029972; H141, MW029973; H145, MW029974; H159, MW029975; H160, MW029976) using Clustal X. The potential recombinant events were preliminarily scanned using Recombinant Detection Program (RDP) v4.0 [24], and further investigated by similarity plot and bootscanning analyses in Simplot v3.5.1 [25]. Phylogenetic origin of the major or minor parental

regions of NOMV strains from patients were constructed from the essential ORFs of the major or minor parental regions of T43. ML phylogenetic trees of the three genome regions between the estimated breakpoints depend on the seven NOMV strains were constructed by MEGA 5.0 to understand the evolutionary relationship of NOMV[26].

Serological analysis

Patients' serum was tested by ELISA using recombinant nucleoprotein (N). The N gene was cloned into pET-30a vector (Novagen) and its induced expression was conducted according to the routine methods. The N protein was purified and confirmed with an anti-His-tag antibody. NOMV-specific IgM and IgG antibodies were detected in patients as described elsewhere[27]. Briefly, the coating antigen was the expressed N protein (5 ng/μL), serial dilutions of patient serum (starting at 1:10) by a factor of two were incubated in an indirect ELISA. The cutoff value of the reaction was calculated as the mean OD of 200 healthy blood donors' sera OD values plus two standard deviations. A sample that showed higher OD value than cutoff line with a titer of ≥ 1:10 was considered NOMV serological IgM or IgG positive.

Neutralizing antibodies for NOMV in serum samples were tested by microneutralization test (MNT), serial dilutions of serum samples (starting at 1:10) by a factor of two were mixed with an equal volume of approximately 100 FFUs NOMV and incubated at 37°C for 1.5 h. The mixture was then added to a 96-well plate in quadruplicate, which was seeded with 1×10^4 BHK-21 cells 12 h before infection. After 3 days incubation, viral infection was detected by IFA using serum samples from patients with laboratory-confirmed infection. The end-point titer was defined as the reciprocal of the highest dilution of serum that prevented infection.

Immunofluorescence assay (IFA) was used to detect the infection of NOMV in different cell lines. Vero, BHK-21, SMMC-7721, WISH, and Caco-2 cells were infected with NOMV at a MOI of 5 in triplicate for 48 h. After fixed with methanol and acetone (1:1), the cells were washed with 1×PBS three times and blocked with 5% BSA for 1 h. The convalescent serum specimens with a dilution of 1:20 were added and incubated for 1 h and probed with fluorescein isothiocyanate–conjugated goat anti-human IgG for another hour. The cellular nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) for 5 min.

Data analysis

The complete sequences of viruses were analyzed by ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder) to find putative viral proteins[28]. The prevalence of pooled samples were calculated by maximum likelihood estimation (MLE) using the program PooledInfRate[29], and the infection rates were statistically compared using either the Chi-square test or Fisher's exact test. Values of $P< 0.05$ were considered as statistically significant.

References

1. https://talk.ictvonline.org/taxonomy (accessed October 6, 2020)

2. Lefkowitz, E.J., et al. Virus taxonomy: the database of the International Committee on Taxonomy of Viruses (ICTV). *Nucleic Acids. Res.* **46**, D708-717 (2018).
3. Tokarz, R., et al. Identification of novel viruses in *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis* ticks. *mSphere* **3**, 00614 – 17 (2018).
4. Li, C.X., et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife* **4**, e05378 (2015).
5. Shi, M., et al. Redefining the invertebrate RNA virosphere. *Nature* **540**, 539–543 (2016).
6. Han, X., et al. Leafhopper *Psammotettix alienus* hosts chuviruses with different genomic structures. *Virus Res.* **285**, 197992 (2020).
7. Maia, L.M.S., et al. Novel viruses in mosquitoes from Brazilian Pantanal. Viruses **11**, 957 (2019).
8. Gong, S. et al. Nairobi sheep disease virus RNA in ixodid ticks, China, 2013. *Emerg. Infect. Dis.* **21**, 718–720 (2015).
9. Argenta, F.F., et al. Identification of reptarenaviruses, hartmaniviruses, and a novel chuvirus in captive native Brazilian *Boa Constrictors* with boid inclusion body disease. *J. Virol.* **94**, 01–20 (2020).
10. Harvey, E., et al. Extensive diversity of RNA viruses in Australian ticks. *J. Virol.* **93**, 01358–18 (2019).
11. Viljakainen, L., et al. Identification and characterisation of common glow-worm RNA viruses. *Virus Genes* **56**, 236–248 (2020).
12. Temmam, S., et al. Monitoring silent spillovers before emergence: A pilot study at the tick/human interface in Thailand. *Front. Microbiol.* **10**, 2315 (2019).
13. Hang, J., et al. Genome sequences of five arboviruses in field-captured mosquitoes in a unique rural environment of South Korea. *Genome Announc.* **4**, e01644-15 (2016).
14. Achazi, K., et al. Detection and differentiation of tick-borne encephalitis virus subtypes by a reverse transcription quantitative real-time PCR and pyrosequencing. *J. Virol. Methods* **171**, 34–39 (2011).
15. Liu, H., et al. The first molecular evidence of severe fever with thrombocytopenia syndrome virus in ticks in Jilin, Northeastern China. *Ticks Tick Borne Dis.* **7**, 1280–1283 (2016).
16. Wang, Z.D., et al. A new segmented virus associated with human febrile illness in China. *N. Engl. J. Med.* **380**, 2116–2125 (2019).
17. Bonczek, O., et al. Identification of *Borrelia burgdorferi* genospecies isolated from *Ixodes ricinus* ticks in the South Moravian region of the Czech Republic. *Ann. Agric. Environ. Med.* **22**, 637–641 (2015).
18. Jiang, B.G., et al. *Borrelia miyamotoi* infections in humans and ticks, Northeastern China. *Emerg. Infect. Dis.* **24**, 236–241 (2018).
19. Wei, F., et al. Molecular detection and characterization of zoonotic and veterinary pathogens in ticks from Northeastern China. *Front. Microbiol.* **7**, 1913 (2016).
20. Liu, H., et al. Characterization of rickettsiae in ticks in northeastern China. *Parasit. Vectors* **9**, 498 (2016).
21. Chen, Z., et al. Ticks (Acari: Ixodoidea: Argasidae, Ixodidae) of China. *Exp. Appl. Acarol.* **51**, 393–404 (2010).

22. Bozzola, J.J. Conventional specimen preparation techniques for transmission electron microscopy of cultured cells. *Methods Mol. Biol.* **1117**, 1–19 (2014).
23. Payne, A.F., et al. Quantitation of flaviviruses by fluorescent focus assay. *J. Virol. Methods* **134**, 183–189 (2006).
24. Martin, D.P., et al. RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics* **26**, 2462–2463 (2010).
25. Zhou, P., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270–273 (2020).
26. Qin, X.C., et al. A tick-borne segmented RNA virus contains genome segments derived from unsegmented viral ancestors. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 6744–6749 (2014).
27. Liu, X., et al. A tentative *Tamdy Orthonairovirus* related to febrile illness in Northwestern China. *Clin. Infect. Dis.* **70**, 2155–2160 (2020).
28. Lu, S., et al. CDD/SPARCLE: the conserved domain database in 2020. *Nucleic Acids Res.* 48, D265-268 (2020).
29. Biggerstaff, B., PooledInfRate software. *Vector Borne Zoonotic Dis.* **5**, 420–421 (2005).

Figures

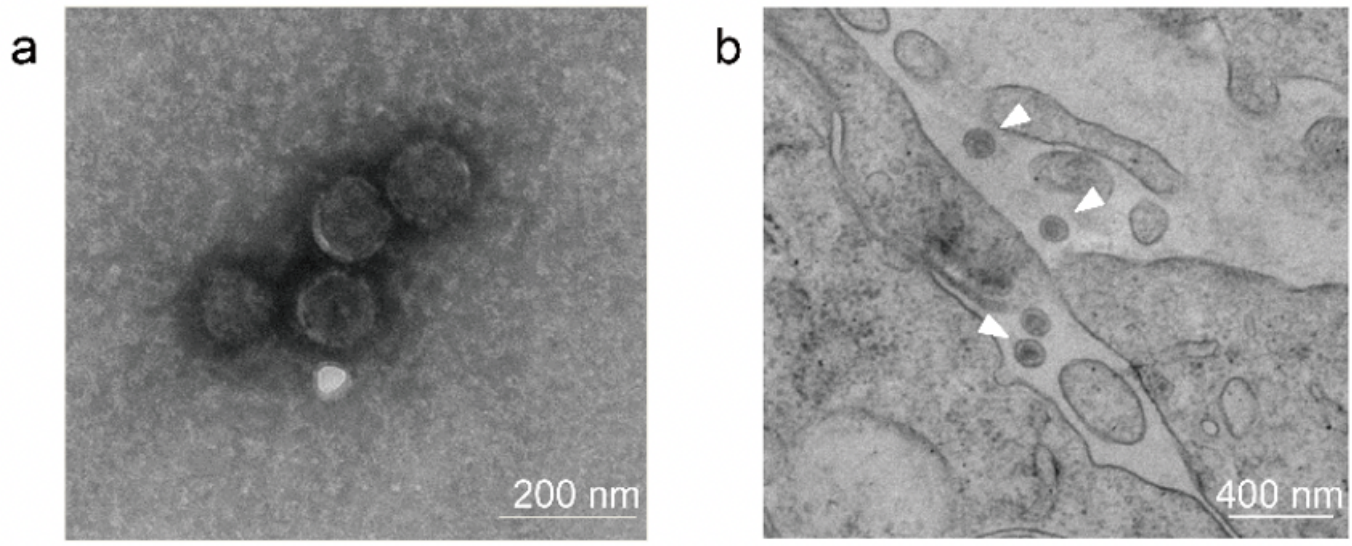

Figure 1

Electron microscopic examination of the new chuvirus. a, Negatively stained virions purified from infected BHK-21 cells. b, Viral particles in the cytoplasmic vacuoles of infected BHK-21 cells.

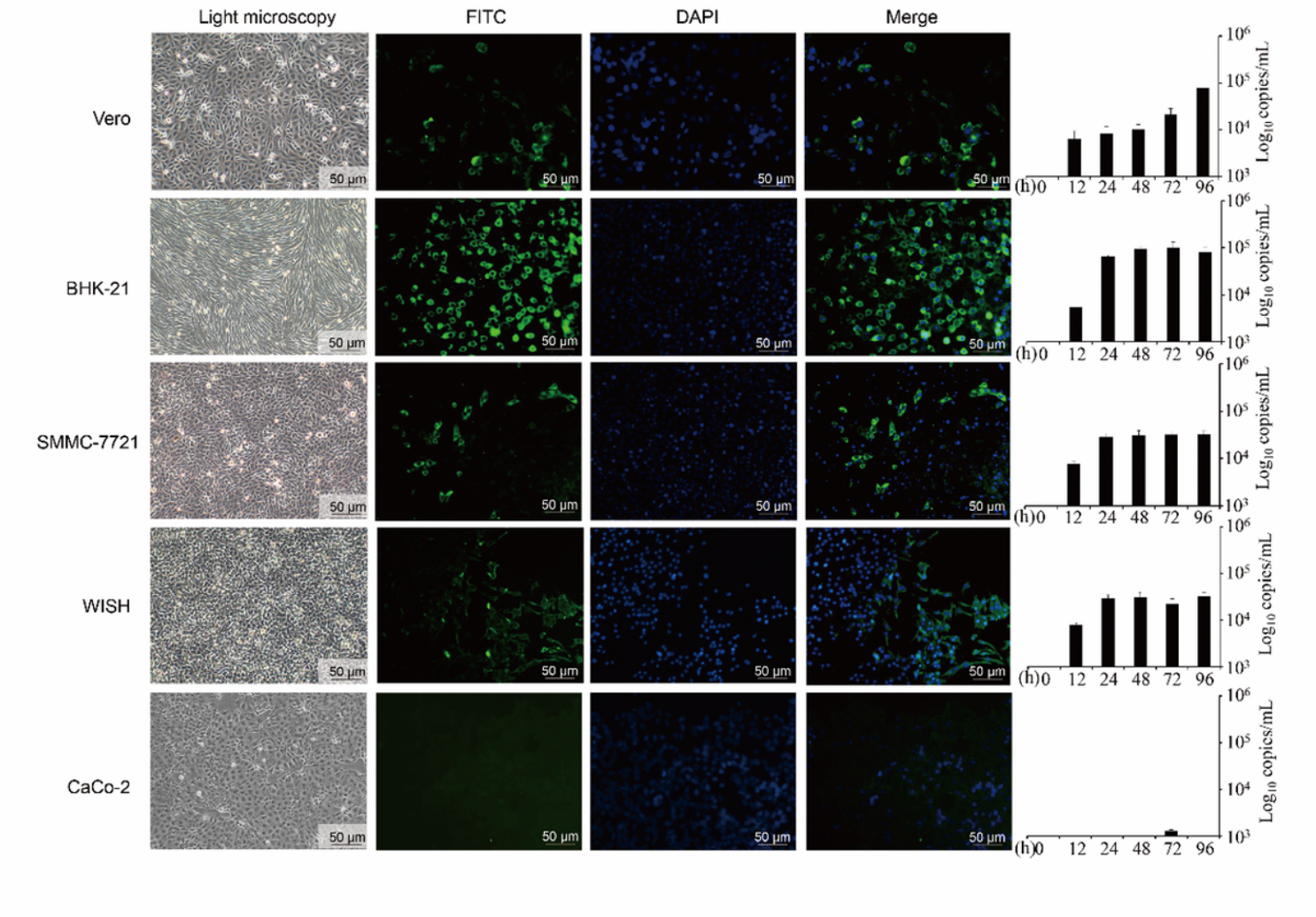

Figure 2

Viral infection in different cells. Vero, BHK-21, SMMC-7721, WISH, and Caco-2 cells were infected with NOMV at a MOI of 5 in triplicate for 48 h and detected by IFA with positive human serum. The supernatants of infected cells were collected for viral quantification at the indicated time points by real-time RT-PCR; Results from three technical replicates were averaged and error bars indicate standard deviation across biological replicates.

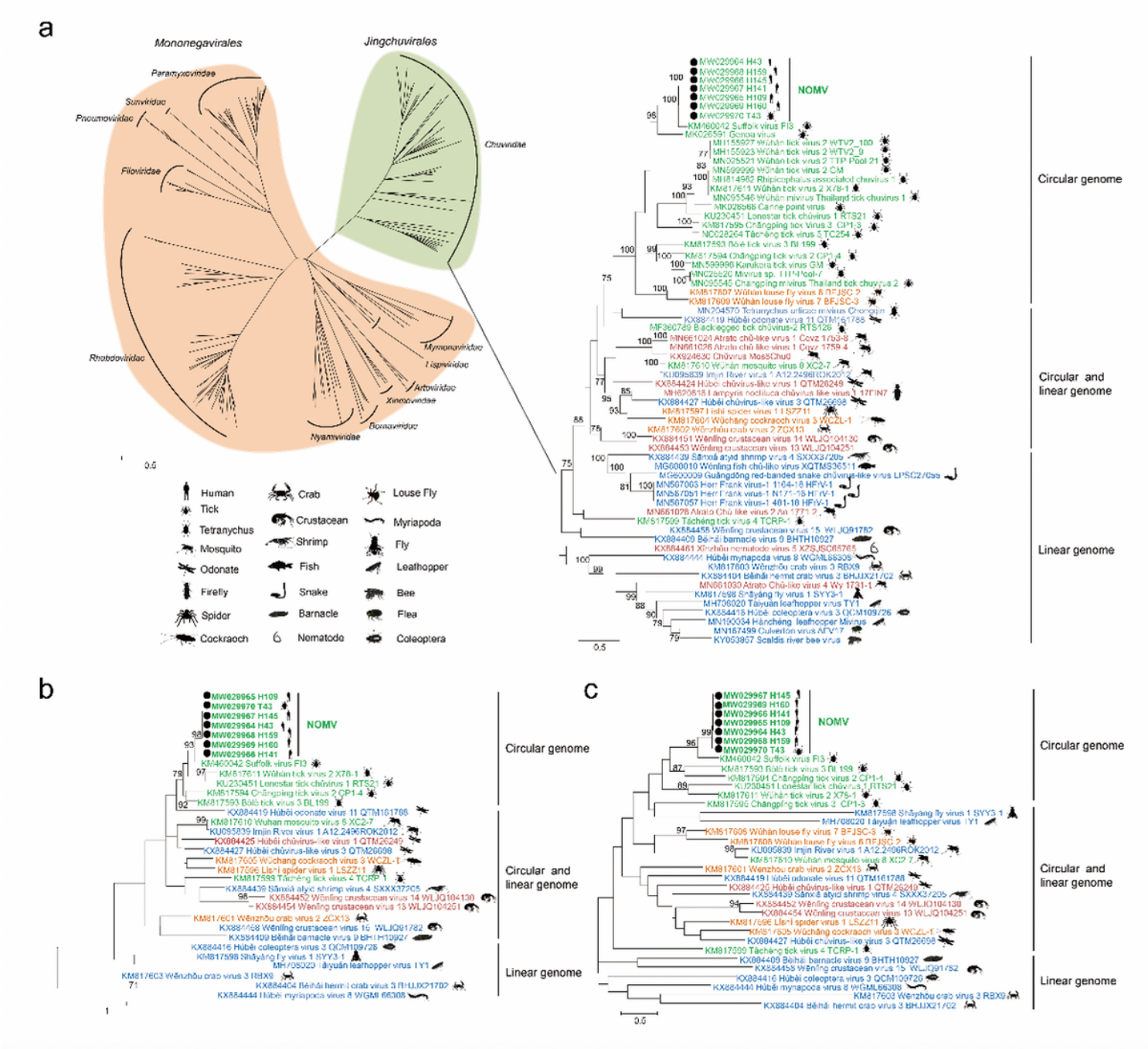

Figure 3

Phylogenetic analysis of NOMV. a, Phylogenetic tree based on the L protein amino acid sequences of NOMVs (black dots) and the typical viruses in the orders Mononegavirales and Jingchuvirales. The clade of the family Chuviridae is extended and shown with rectangular tree. b, Phylogenetic tree based on the complete G protein amino acid sequences of NOMVs and other chuviruses. c, Phylogenetic analysis based on the complete N protein amino acid sequences of NOMVs and other chuviruses. The trees were constructed by the MEGA 5.0 software with the maximum likelihood method and Jones-Taylor-Thornton model. Bootstrap values higher than 70% are shown. Chuviruses with circular, bi-circular, unsegmented, and bi-segmented genome are shaded green, orange, blue, and red, respectively. The silhouette figures represent the virus host. The abbreviations of virus names are shown in Supplemental Tables S2–S4.

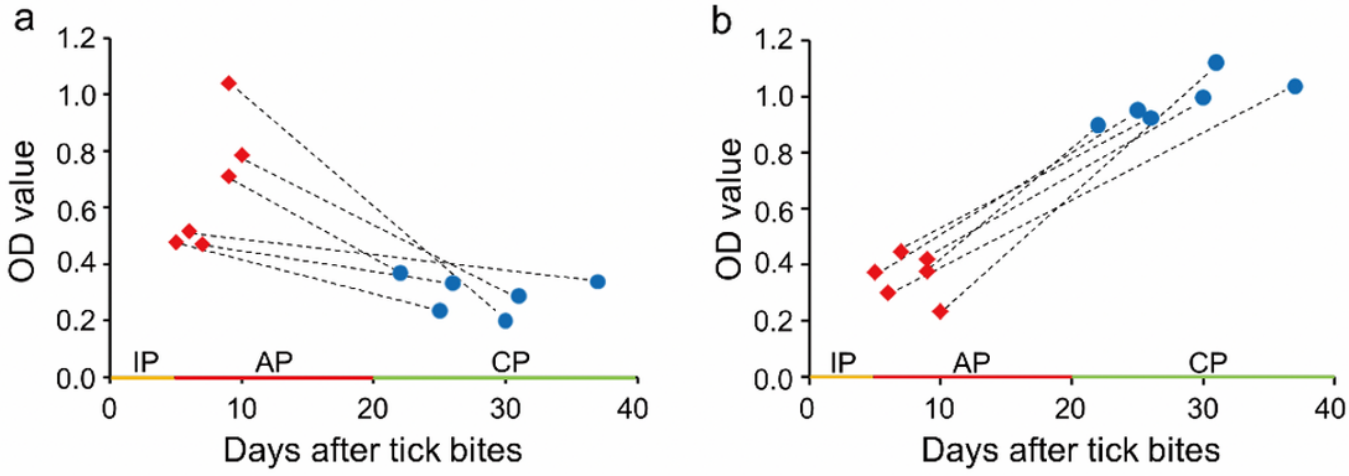

Figure 4

Serological investigation of NOMV patients. Virus-specific IgM (a) and IgG (b) antibodies in six patients were detected by the N protein-based ELISA. The red rhombuses and blue dots indicate the OD values of each patient in the acute period (AP) and convalescent period (CP), respectively (More information can be found in Extended Data Table 4). The black dotted line matches the OD value of the same patient in AP and CP. The cutoff value was set up as 0.38 for IgM test and 0.40 for IgG test according to healthy controls.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementdata20201120177.docx
- ExtendedData20201120177.pdf