

# Metagenomic Analysis of Tick-Borne Viruses in Hulunbuir, Inner Mongolia, China: Epidemiological Risk of Potential Novel Pathogenic Viruses Relevant to Public Health

# Metagenomic Analysis of Tick-Borne Viruses in Hulunbuir, Inner Mongolia, China: Epidemiological Risk of Potential Novel Pathogenic Viruses Relevant to Public Health

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## Abstract

**Objective:** Inner Mongolia, with its vast grasslands providing ideal habitats for ticks, has recently emerged as a hotspot of novel tick-borne virus (TBV) prevalence. However, the diversity of TBVs and their potential spillover risks remain to be elucidated.

**Methods:** In 2021, we collected 500 *Haemaphysalis longicornis*, *Ixodes persulcatus*, and *Dermacentor silvarum* ticks from Hulunbuir, Inner Mongolia, and conducted virome analysis and viral surveillance to evaluate TBV exposure risk in Hulunbuir.

**Results:** A total of 26 viral species belonging to nine families were identified, including four novel viruses (Yilieked tick virus 1 [YLTV1, *Rhabdoviridae*], Meitian tick virus [MtTV, *Phenuiviridae*], Wunuer pestivirus, and Wunuer tick phlebovirus) and 11 new strains of human-pathogenic viruses (including Songling virus [SGLV], Beiji nairovirus [BJNV], and Nuomin virus [NOMV]), from three dominant tick species. Virome profiling revealed striking species-specific patterns: *I. persulcatus* exhibited the highest viral richness and abundance, dominated by *Chuviridae* (NOMV) and *Phenuiviridae*; *D. silvarum* uniquely carried *Phenuiviridae* (MtTV); and *H. longicornis* showed *Nairoviridae* predominance (SGLV, BJNV). Phylogenetic analyses demonstrated that MtTV clustered with human-pathogenic Tacheng tick virus 2, and NOMV-YL strains showed high similarity to clinical NOMV isolates from febrile patients. Regional surveillance demonstrated MtTV's pan-regional circulation in *D. silvarum* across nine locations (MIR=4–8%), contrasting with localized Yilieked foci for SGLV (MIR=0.6%), BJNV (0.4%), and NOMV (1.0%).

**Conclusions:** This study not only mapped the landscape of TBVs in a key pastoral region, but also highlighted the potential spillover risk of TBVs, including MtTV, YLTV1, SGLV, BJNV, and NOMV. Our findings underscore the urgent need for One Health strategies for monitoring emerging TBVs at the tick-human-livestock interface in northern China.

**Key words:** Tick-borne virus, metagenomics, virome, epidemical investigation, ecological distribution, Hulunbuir

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## INTRODUCTION

The rapid emergence and spread of severe infections with viruses including Zika virus [1], dengue virus [2], yellow fever virus [3], and Crimean–Congo hemorrhagic fever virus (CCHFV) [4] have highlighted the vulnerability of global populations to the possible health and economic consequences of vector-borne viruses. Ticks are an important vector transmitting viral pathogens associated with human and animal diseases, and playing critical roles in viral dissemination. Some of the most notoriously pathogenic TBVs, including CCHFV [4], tick-borne encephalitis virus (TBEV) [5], severe fever with thrombocytopenia syndrome virus (SFTSV) [6], Nairobi sheep disease virus (NSDV) [7], and African swine fever virus (ASFV) [8], have exerted notable public health and economic effects. Tick-borne viruses (TBVs) are increasingly emerging on a global scale, and posing significant threats to both human and animal health [9].

Inner Mongolia, a vast and diverse region in China, has recently emerged as a hotspot of novel TBVs, including Alongshan virus (ALSV) [10], Songling virus (SGLV) [11], Wetland virus (WELV) [12], Tacheng tick virus 2 (TcTV2) [13], and Beiji nairovirus (BJNV) [14]. The emergence of these TBVs is a growing global public health concern, because of their pathogenicity and potential to cause severe illness in humans. ALSV, a novel segmented Flavivirus, was first detected in patients with febrile illness in Inner Mongolia and Heilongjiang, and natural infections in livestock were observed in Hulunbuir, Inner Mongolia [10,15]. Similarly, the newly identified SGLV was isolated from patients in Yakeshi, Inner Mongolia in 2017 [11]. Moreover, re-emergence of globally circulating viruses such as CCHFV and TBEV has also been reported in Inner Mongolia [10,11,16,17]. CCHFV has been identified in *Hyalomma* spp. from the Alashan region in Inner Mongolia [16], and positive serological evidence in camels (58.3%) and sheep (6.7%) has suggested potential risk to the human population, although no recent human cases have been reported [17]. TBEV has been identified in circulation among ticks (*Ixodes persulcatus* and *Dermacentor* spp.) and humans in Inner Mongolia [18]. Therefore, Inner Mongolia might be at significant risk of TBV spread.

Beyond these known pathogenic viruses, unclassified viruses such as Bole tick virus (BLTV4), South Bay virus, Norwegian nairovirus 1, and Gakugsa tick virus have been found in Inner Mongolia [19–21]. These newly discovered viruses have prompted further concerns regarding potential public health threats, because their pathogenicity remains unknown. These discoveries underscore the need for continued surveillance and research, and highlight the complexity of the TBV landscape in Inner Mongolia.

The distribution of tick species in Inner Mongolia, particularly in the Hulunbuir region, is significant. Species of the genus *Dermacentor*, including *D. nuttalli* and *D.*

*silvarum*, are distributed mainly in arid grassland areas suitable for grazing cattle and sheep [22]. Hulunbuir, an important pastoral area in Inner Mongolia known for its vast grasslands, is a notable livestock production area and an essential habitat for *Dermacentor* and *Ixodes* genera [22]. Several tick-borne pathogens reported in the Hulunbuir region pose severe threats to human and animal health.

In this study, we collected *Dermacentor silvarum*, *Ixodes persulcatus*, and *Haemaphysalis longicornis* tick samples across ten towns in the Inner Mongolia Autonomous Region, with the aim of investigating the diversity and ecological characteristics of TBVs. We identified two novel viruses, Yiliekedek tick virus1 (YLTV1) and Meitian tick virus (MtTV), belonging to the *Rhabdoviridae* and *Phenuiviridae* families, respectively. Given the potential of these viruses to cause human infectious diseases, we conducted a survey of SGLV, BJNV, YLTV1, and MtTV Nuomin virus (NOMV) prevalence within the tick population, to assess the exposure risks in Hulunbuir region. This research not only markedly advances understanding of TBV transmission dynamics and potential health threats in northern China, but also provides essential insights for the development of effective prevention and control strategies in this region, which is a hotspot of TBV prevalence.

## MATERIALS AND METHODS

### Sample collection and tick species identification

A total of 500 ticks were collected from ten towns (Yiliekedek, Wunuer, Meitian, Dayan, Mianduhe, Moguai, Muyuan, Tenihe, Yakeshi, and Zhaluomude) in Inner Mongolia Province, China, in 2021 (S1 Table and Fig 1). All ticks were collected from the tips of grass blades and stored on dry ice before transport to the laboratory, to ensure preservation of viral activity. After arrival at the laboratory, tick samples were directly preserved in a  $-80^{\circ}\text{C}$  freezer for long-term storage. All ticks were identified morphologically according to species and life stage, with existing taxonomic keys and a Nikon SMZ445 stereomicroscope. After morphological assessment, selected tick specimens representing diverse areas, hosts, and species were subjected to molecular analysis. Each tick was individually rinsed with PBS, then homogenized in a solution containing 180  $\mu\text{L}$  ATL buffer and 20  $\mu\text{L}$  Proteinase K with a Tissue Cell-destroyer (Novastar, China). DNA was extracted with a QIAamp Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed on individual specimens of each tick species to amplify an 820 bp amplicon of the mitochondrial *cytochrome c oxidase subunit I* gene, with optimized conditions and primers specific as previously described [23]. The PCR products were purified with a QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany) and subsequently subjected to bidirectional Sanger sequencing (Sangon Biotech, Shanghai, China).

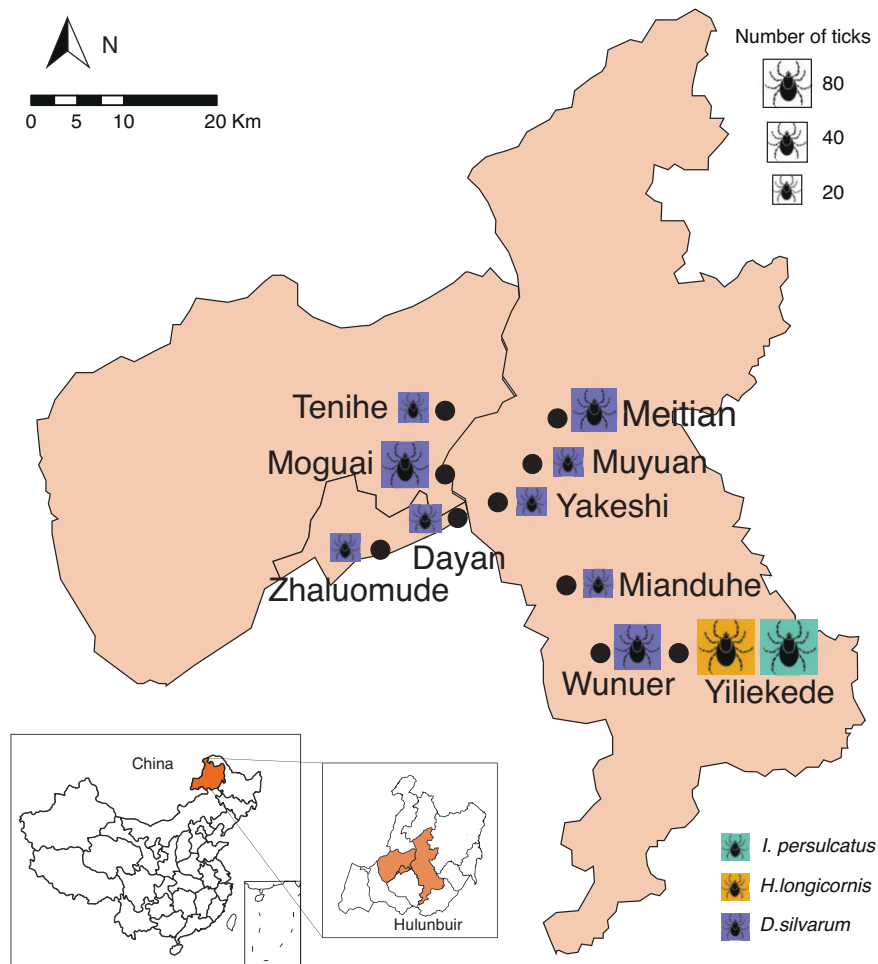


**FIGURE 1** | Map of tick sampling locations and tick species in the Hulunbuir area.

Map of China, showing the location of Hulunbuir in Inner Mongolia Province, where sampling occurred (colored orange). The tick sampling collection locations are marked with solid black dots on the map. Tick species collected from each sampling location are color coded as follows: blue for *I. persulcatus*, yellow for *H. longicornis*, and purple for *D. silvarum*.

### Tick sequencing library construction and sequencing

A total of 12 tick sequencing libraries were generated according to the sampling location, tick species, and sex (S2 Table). Ticks from the same library were washed three times with sterile, RNA- and DNA-free phosphate-buffered saline (PBS, HyClone™), then roughly minced with sterile tweezers and surgical scissors, and homogenized in PBS solution with a Tissue Cell-destroyer (Novastar, China). The homogenate was incubated at 55°C for 10 minutes with Proteinase K (Qiagen) and centrifuged at 15,000 *g* for 30 seconds. The supernatant was collected for total RNA extraction with TRIzol (Life Invitrogen™, Carlsbad, CA, USA). For each pool, 1 µg RNA was used for library preparation and subjected to RNA-Seq with a HiSeq 3000 sequencer according to the

manufacturer's instructions (Illumina, San Diego, USA), as previously described [24]. The quantity of extracted RNA was measured with a Qubit 4.0 fluorometer, and RNA quality was assessed with an Agilent Bioanalyzer 2200 (Agilent). The RNA library underwent paired-end sequencing, with a 10 G sequence depth per library and a read length of 150 base pairs per end, with the HiSeq 3000 sequencing platform (Illumina, San Diego, CA, USA). This high-throughput sequencing was performed at Novogene Technologies.

### Tick virome analysis and virus discovery

The raw sequencing data were processed to remove low-quality reads, adapter sequences, and contaminants. Trimmomatic (version 0.40) was used to trim and filter the reads according to quality scores. Furthermore, FastQC

(version 0.11.9) was used to assess read quality. Bowtie2 (version 2.3.3.1) was used to extract and filter the tick-related reads according to the *Ixodoidea* database from the National Center for Biotechnology Information (NCBI) [25]. The remaining sequencing reads were assembled in Trinity (version 2.5.1) [24]. All assembled contigs longer than 200 base pairs were subjected to BLASTn analysis against all non-redundant nucleotide databases with the local BLAST tool [26], and BLASTx analysis against all non-redundant protein databases obtained from GenBank with DIAMOND (version 0.9.24). Any result with an e-value below  $1 \times 10^{-6}$  was considered a “significant hit.” All putative viral contigs exhibiting overlapping regions exceeding 100 bp and a threshold value of 95% similarity were initially merged with the SeqMan program in the Lasergene package (DNASTar version 7.1). All filtered viral contigs were annotated and classified according to the top hit from BLASTx comparisons. If a contig showed no homology in the BLASTx results, it was then classified according to the best match from BLASTn. A novel virus was proposed if the amino acid (aa) similarity with respect to the RdRp domain was below 90%, as subsequently confirmed by phylogenetic analyses [27,28].

All ribosomal reads in each library were mapped to the rRNA database (<https://www.arb-silva.de/>) with Bowtie2 (version 2.4.3), to avoid possible effects of unequal rRNA removal efficiency during sequencing library preparation. The non-rRNA reads in each library were then aligned to the assembled sequences with the Bowtie2 end-to-end alignment method with sensitive parameters. After the removal of rRNA reads, transcripts per million were calculated and used to standardize the relative abundance of each virus. A heatmap was constructed to visualize the profile of the tick-related virome with the heatmap package in R Studio.

### Annotation of viral genomes

Viral ORFs were predicted with ORF Finder (<https://www.ncbi.nlm.nih.gov/Orffinder/>). All predicted ORFs were longer than 100 aa. Only the longest ORF was reported if a shorter ORF was completely nested within it. All predicted ORFs were annotated by alignment with the BLASTp non-redundant protein database and the PSI-BLAST conserved domain database (e-value threshold of  $1 \times 10^{-5}$ ). Read coverage and continuity of novel viral genomes were verified with Bowtie2.

### Genome and phylogenetic analysis

The highly conserved RdRp gene or NS5 gene (including RdRp gene) was selected to construct the family- and order-level phylogenies. The phylogenetic clades were divided at the genus level. Novel viruses and new viral strains identified in this study were aligned with representative reference sequences belonging to the corresponding viral family downloaded from GenBank, including *Flaviviridae*, *Phenuiviridae*, *Chuviridae*, *Rhabdoviridae*, and *Nairoviridae*. Phylogenetic trees were constructed with the maximum

likelihood method and GTR+G model in MEGA (version 7.0), with 1,000 bootstrap replicates.

### Virus classification

According to the taxonomy disciplines from the International Committee on Taxonomy of Viruses, on the basis of the nt and aa sequence identities, a novel virus was defined when it had an aa sequence identity of <90% with respect to the reference virus RNA-dependent RNA polymerase (RdRp), or a genomic sequence with <80% identity with respect to the reference virus [28,29]. The presence of viruses identified from metagenomic sequencing was further validated with PCR. Primers were designed on the basis of the conserved regions identified in the metagenomic analysis (S3 Table). PCR amplification was performed in 25  $\mu$ L reaction mixtures containing 12.5  $\mu$ L 2 $\times$ PCR Master Mix (Promega, USA), 1  $\mu$ L of each primer (10  $\mu$ M), 1  $\mu$ L cDNA template obtained by reverse transcription from RNA libraries, and 8.5  $\mu$ L nuclease-free water. The cycling conditions were as follows: 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute; and 72°C for 5 minutes. The PCR products were verified with gel electrophoresis and further confirmed through Sanger sequencing (Sangon Biotech, Shanghai, China).

### Detection of SGLV, BJNV, YLTV1, MtTV, and NOMV among tick groups

A total of 340 *D. silvarum*, 80 *I. persulcatus*, and 80 *H. longicornis* tick individuals were grouped into 32 pools to investigate the prevalence of viruses, including SGLV, BJNV, YLTV1, MtTV, and NOMV, identified among tick individuals in Inner Mongolia. Specific nested reverse transcription-polymerase chain reaction (nested PCR) primers were designed on the basis of the integrity of the viral genomes. The primers used for detection are listed in S3 Table. Tick group RNA extraction and viral detection were performed, as previously described [23]. All positive amplicons detected in this study were sequenced with Sanger sequencing (Sangon Biotech, Shanghai, China). We calculated the minimum infectivity rate (MIR) to identify the prevalence of viral infections among studied tick populations. The MIR is the ratio of the number of positive pools to the total number of tick individuals tested. This metric is frequently used to estimate the proportion of infected ticks within a sample and is calculated as follows:

$$MIR = \left( \frac{\text{Number of infected tick pools}}{\text{Total number of ticks individuals tested}} \right) \times 100.$$

To quantify the uncertainty associated with the MIR estimates, we calculated 95% confidence intervals (CIs) as follows:

$$CI = \left[ p \pm Z_{\frac{\alpha}{2}} \times \sqrt{\frac{p(1-p)}{n}} \right],$$

where  $p$  is the proportion of infected ticks, calculated as follows:

$$p = \frac{\text{Number of infected tick pools}}{\text{Total number of ticks individuals tested}},$$

$Z_{\alpha/2}$  is the critical value for a 95% CI (approximately 1.96), and  $n$  is the total number of samples tested.

## RESULTS

### Overall virome in three dominant tick species in Hulunbuir

We constructed and sequenced 12 RNA libraries containing 40 *D. silvarum* ticks from Meitian and Wunuer; 80 *I. persulcatus* ticks from Yiliegede; and 80 *H. longicornis* ticks from Yiliegede in Hulunbuir during 2021 (S1 Table and Fig 1). A total of 87,775,647–150,413,092 raw sequencing reads per library were obtained after trimming and cleaning with Bowtie2 v2.4.3. After removal of tick-related reads, the remaining reads were subjected to de novo assembly and alignment with the non-redundant nt and nr databases from NCBI with BLASTn, BLASTx, and DIAMOND. A total of 2,843–297,444 reads, representing 0.0014–0.2883% of the total sequencing reads within each tick library, were mapped to virus and unassigned-virus. Details of the tick sequencing libraries are summarized in S2 Table. All virus-related reads across 12 tick libraries from Inner Mongolia were annotated into at least 26 viral species within nine families: *Chuviridae*, *Coronaviridae*, *Flaviviridae*, *Luteoviridae*, *Nairoviridae*, *Partitiviridae*, *Phenuiviridae*, *Rhabdoviridae*, and *Totiviridae* (Table 1).

### Virome patterns associated with tick species in Hulunbuir

The numbers of reads mapped to viruses and proportion of viral reads among three tick species libraries are shown in Fig 2A and B and S2 Table. In the *I. persulcatus* tick libraries, 119,993 to 297,444 reads per sample were annotated to three to seven different viral species, whereas 2,843 to 67,447 reads in *H. longicornis* tick libraries were annotated to two to six viral species; 6,071 to 37,338 aligned reads in *D. silvarum* libraries were identified to be related to four or five viral species. Moreover, the number and proportion of viral reads was significantly higher in the *I. persulcatus* tick libraries than the *D. silvarum* and *H. longicornis* tick libraries (Fig 2A and B). Virome composition is presented at the virus family level among the three tick species (Fig 2C). Members of the *Flaviviridae* and *Phenuiviridae* were more frequently detected in *D. silvarum* ticks; *Chuviridae* were more frequently detected in *I. persulcatus* ticks; and *Nairoviridae* were more frequently detected in *H. longicornis* ticks. Rarefaction curve analysis of viral species richness in each tick library showed that the sequencing depth of all pools was sufficient (Fig 2D). The alpha diversity analysis indicated that both Shannon indices were higher in *D. silvarum* and *I. persulcatus* than in *H. longicornis*, thus suggesting a higher viral diversity in the former than the latter, although the difference was not

significant (Fig 2E). Nevertheless, beta diversity analysis (NMDS) revealed a significant difference in viral species (stress=0.007,  $p=0.002$ ), whereas geographic distribution and tick sex did not significantly affect tick virome diversity (Fig 2G). Furthermore, we explored the virome pattern between *D. silvarum*, *I. persulcatus*, and *H. longicornis* according to the log<sub>2</sub> normalized abundance of 26 eukaryotic viral species (rows) across the 12 libraries (Fig 2C). The viromes from the same tick species libraries clearly clustered separately, according to hierarchical clustering based on the Euclidean distance matrix. Specifically, most *I. persulcatus* ticks carried NOMV, Norway nomonegavirus 1, Beiji nairovirus, Mukawa virus, Norway phlebovirus, and Sara tick phlebovirus in high abundance. However, reads associated with SGLV and Wuhan insect virus 7 were highly abundant in *H. longicornis* ticks. Some viruses, most related to bole tick virus 4 and Tacheng tick virus 2, were uniquely present in *D. silvarum* ticks. In addition, the viral distribution and abundance exhibited no significant preference for tick sex and location in this study. Similar virome distribution characteristics were observed between *I. persulcatus* and *H. longicornis* ticks from Yiliegede. Moreover, the detected *D. silvarum* ticks from Wunuer and Meitian showed highly similar predominating virome constructs. These results indicated that tick species plays an important role in shaping the characteristics of the viral spectrum carried by ticks in Hulunbuir.

### Diversity and evolution of novel viruses and viral strains

#### *Chuviridae*

Three NOMV-YL genomes belonging to the newly classified family *Chuviridae* were identified in *I. persulcatus* from Yiliegede, with lengths of 10,874, 11,337, and 11,321 nt. Their genomes comprised three ORFs: RdRp, glycoprotein (GP), and nucleoprotein (NP). NOMV-YL showed the highest sequence similarity to NOMV-H160 (aa identity of 99.5–100%, 99.7–100%, and 99.68–100% to RdRp, GP, and NP, respectively), and was identified from febrile patients according to the GenBank (MW029969). Phylogenetic tree analysis also indicated that NOMV-YL is most closely related to NOMV-H160 (Fig 3).

#### *Flaviviridae*

*Flaviviridae* consist of four genera: Flavivirus, Hepacivirus, Pegivirus, and Pestivirus [30]. BLTV4, an unclassified pestivirus, has recently been defined as having only one ORF that encodes a polyprotein [19]. Three complete genome sequences of a novel pestivirus-like virus were obtained from *D. silvarum* tick species in Meitian and Wunuer, and showed 98.23–99.51% nt identity with one another. These viruses have been tentatively named Wunuer pestivirus, according to the sampling location. The three Meitian pestivirus strains encode an ORF containing 2,207 aa, and are most closely related to BLTV4, with 86.62–87.09% and 86.62–87.09% nt and aa identity, respectively (Fig 3).

**TABLE 1** | Number of virus-related reads identified in tick sequencing libraries and their identity with respect to the closest viral species.

| Family         | Genus                  | Closest viral species              | Meitian           |                   | Wunuer            |                   | Yiliegede          |                    | <i>I. pers</i> (♀) | <i>I. pers</i> (♂) | <i>H. long</i> (♀) | <i>H. long</i> (♂) | <i>H. long</i> (♀) | <i>H. long</i> (♂) |
|----------------|------------------------|------------------------------------|-------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                |                        |                                    | <i>D. sil</i> (♂) | <i>D. sil</i> (♀) | <i>D. sil</i> (♂) | <i>D. sil</i> (♀) | <i>I. pers</i> (♂) | <i>I. pers</i> (♀) |                    |                    |                    |                    |                    |                    |
| Chuviridae     | <i>Chuvirus</i>        | Nuomin virus                       |                   |                   |                   |                   | 51,070             | 234,471            | 53,205             | 9                  |                    |                    |                    |                    |
| Coronaviridae  | <i>Coronavirus</i>     | Longquan R1 rat coronavirus        |                   |                   |                   | 4                 |                    |                    |                    |                    |                    |                    |                    |                    |
|                | Unclassified           | Bole tick virus 4                  | 10,025            | 9,517             | 610               | 4,312             |                    |                    |                    |                    |                    |                    |                    |                    |
| Flaviviridae   | Unclassified           | Norway luteo-like virus            | 5                 |                   |                   |                   |                    | 157,165            | 16                 |                    |                    |                    |                    |                    |
| Luteoviridae   | Unclassified           | Beiji-nairovirus                   |                   |                   |                   |                   | 21,983             |                    | 533                |                    |                    |                    |                    |                    |
| Nairoviridae   | <i>Orthonaurovirus</i> | Songling virus                     |                   |                   |                   |                   |                    |                    |                    | 41,122             | 23,481             |                    | 20,224             |                    |
| Partitiviridae | Unclassified           | Lichen partiti-like RNA virus 2    |                   |                   |                   |                   | 8,597              |                    |                    |                    |                    |                    |                    |                    |
| Phenuiviridae  | <i>Phlebovirus</i>     | Changping tick virus 1             |                   |                   | 1,775             | 392               |                    |                    |                    |                    |                    |                    |                    |                    |
| Rhabdoviridae  | Unclassified           | Beiji phlebovirus                  |                   |                   |                   |                   | 88,777             |                    | 65                 |                    |                    |                    |                    |                    |
|                | Unclassified           | Blacklegged tick phlebovirus       |                   |                   |                   |                   |                    |                    |                    | 17                 |                    |                    |                    |                    |
|                | Unclassified           | Mukawa virus                       |                   |                   |                   |                   |                    |                    |                    | 99,610             |                    |                    |                    |                    |
|                | Unclassified           | Norway phlebovirus                 |                   |                   |                   |                   |                    |                    |                    | 17,638             |                    |                    |                    |                    |
| Totiviridae    | Unclassified           | Omega tick phlebovirus             |                   |                   |                   |                   |                    | 1,422              |                    |                    |                    |                    |                    |                    |
|                | Unclassified           | Sara tick phlebovirus              |                   |                   |                   |                   | 102,307            |                    | 17,679             | 2,170              |                    |                    |                    |                    |
|                | Unclassified           | Tacheng tick virus 2               | 2,121             | 8,136             | 2,368             | 750               |                    |                    |                    |                    |                    |                    |                    |                    |
|                | Unclassified           | American dog tick rhabdovirus 2    |                   | 4,621             |                   |                   |                    |                    |                    |                    |                    |                    |                    |                    |
| Unclassified   | Unclassified           | Nayun tick rhabdovirus NY-13       |                   |                   |                   |                   |                    |                    |                    |                    |                    |                    | 1,953              |                    |
|                | Unclassified           | Taishuan tick virus                |                   | 1,238             |                   |                   |                    |                    |                    |                    |                    |                    |                    |                    |
|                | Unclassified           | Norway mononegavirus 1             |                   |                   |                   |                   | 33,307             |                    | 16,205             |                    |                    |                    |                    |                    |
|                | Unclassified           | Manly virus                        |                   |                   |                   |                   |                    |                    |                    | 23,456             | 2,843              |                    | 1,222              |                    |
| Unclassified   | Unclassified           | Lonestar tick totivirus            |                   |                   |                   | 561               |                    |                    |                    | 115                |                    |                    | 14                 |                    |
|                | Unclassified           | Bawangfen virus                    |                   |                   |                   |                   |                    |                    |                    | 81                 |                    |                    | 59                 |                    |
|                | Unclassified           | Kwi virus                          |                   |                   |                   |                   |                    |                    |                    | 2,673              |                    |                    | 14                 |                    |
|                | Unclassified           | Tick-borne tetraviruses-like virus |                   |                   |                   |                   |                    |                    |                    |                    |                    |                    |                    |                    |
| Subtotal       | Unclassified           | Vovk virus                         |                   |                   |                   |                   |                    |                    |                    | 2,726              |                    |                    |                    |                    |
|                | Unclassified           | Xinjiang tick-associated virus 1   |                   | 1,004             | 1,318             |                   |                    |                    |                    |                    |                    |                    |                    |                    |
|                | Unclassified           |                                    | 37,338            | 24,516            | 6,071             | 10,260            | 297,444            | 247,216            | 191,114            | 119,993            | 67,447             | 2,843              | 25,521             | 21,446             |
|                | Unclassified           |                                    |                   |                   |                   |                   |                    |                    |                    |                    |                    |                    |                    |                    |

*I. pers*, *I. persulcatus*; *D. sil*, *D. silvarum*; *H. long*, *H. longicornis*.

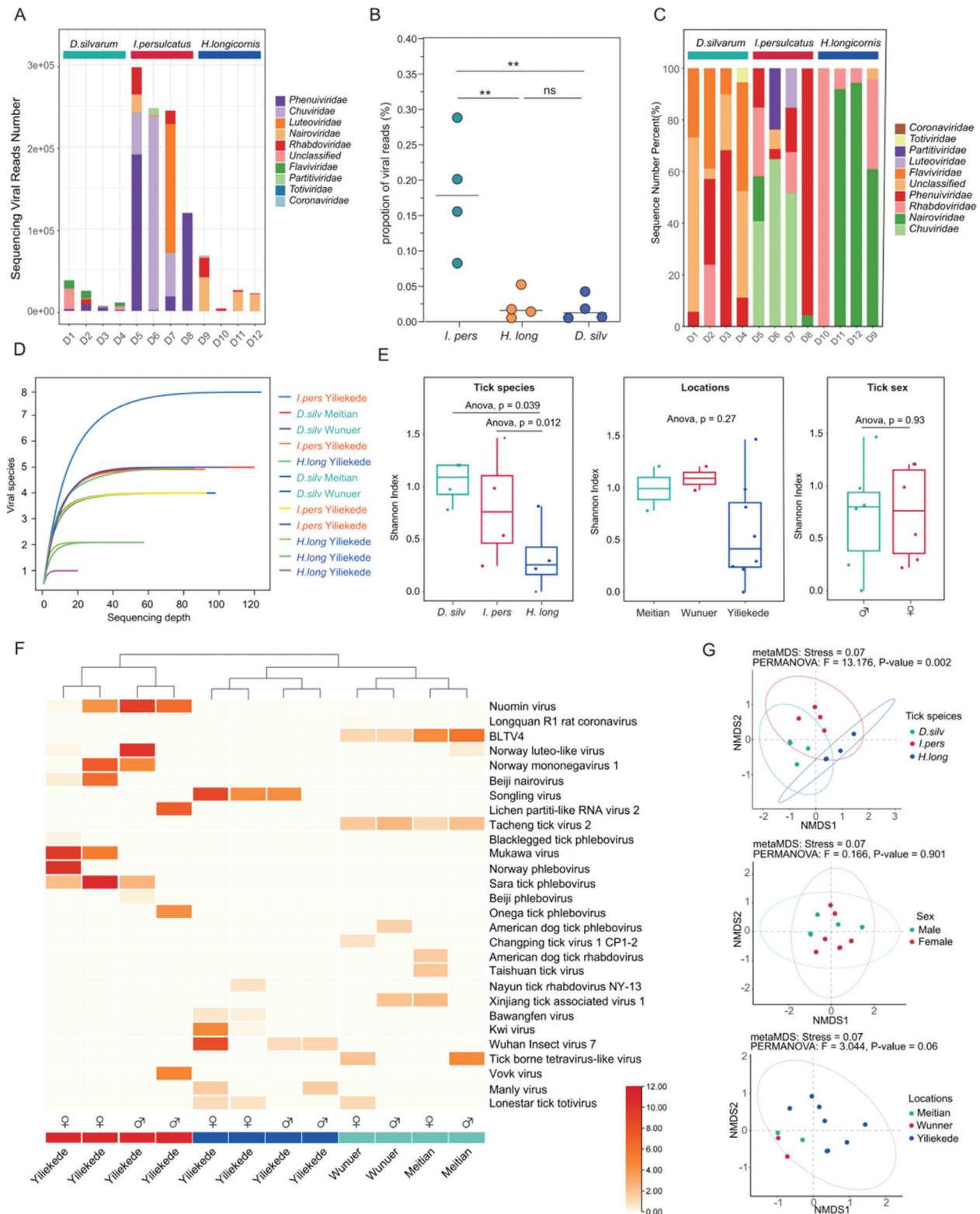


**FIGURE 2 |** Virome distribution pattern among ticks in the Hulunbuir area.

(A) Mapped number of viral reads in each tick library. (B) Comparison of viral read proportions among the three tick species libraries. (C) Virome composition, determined for each tick library and tick species. (D) Rarefaction curves of viral species richness in ticks. The x-axis represents the sequencing depth ( $10^3$  transcripts per million [TPM]), and the y-axis shows the cumulative number of viral species observed. Each curve corresponds to a different sample or group of samples. (E) Alpha diversity of the virome distribution among tick species, locations, and sexes, according to the Shannon index. (F) Normalized abundance of eukaryotic viral species in each tick library, calculated according to TPM on a  $\log_2$  scale. The sampling locations, tick species, and tick sexes are shown at the bottom of the map, and each closest viral species is shown on the right of the heatmap according to the annotation results from BLASTx and DIAMOND. The hierarchical clustering model was based on the calculated Euclidean distance matrix. (G) NMDS analysis of virome distribution by tick species, location, and sex.

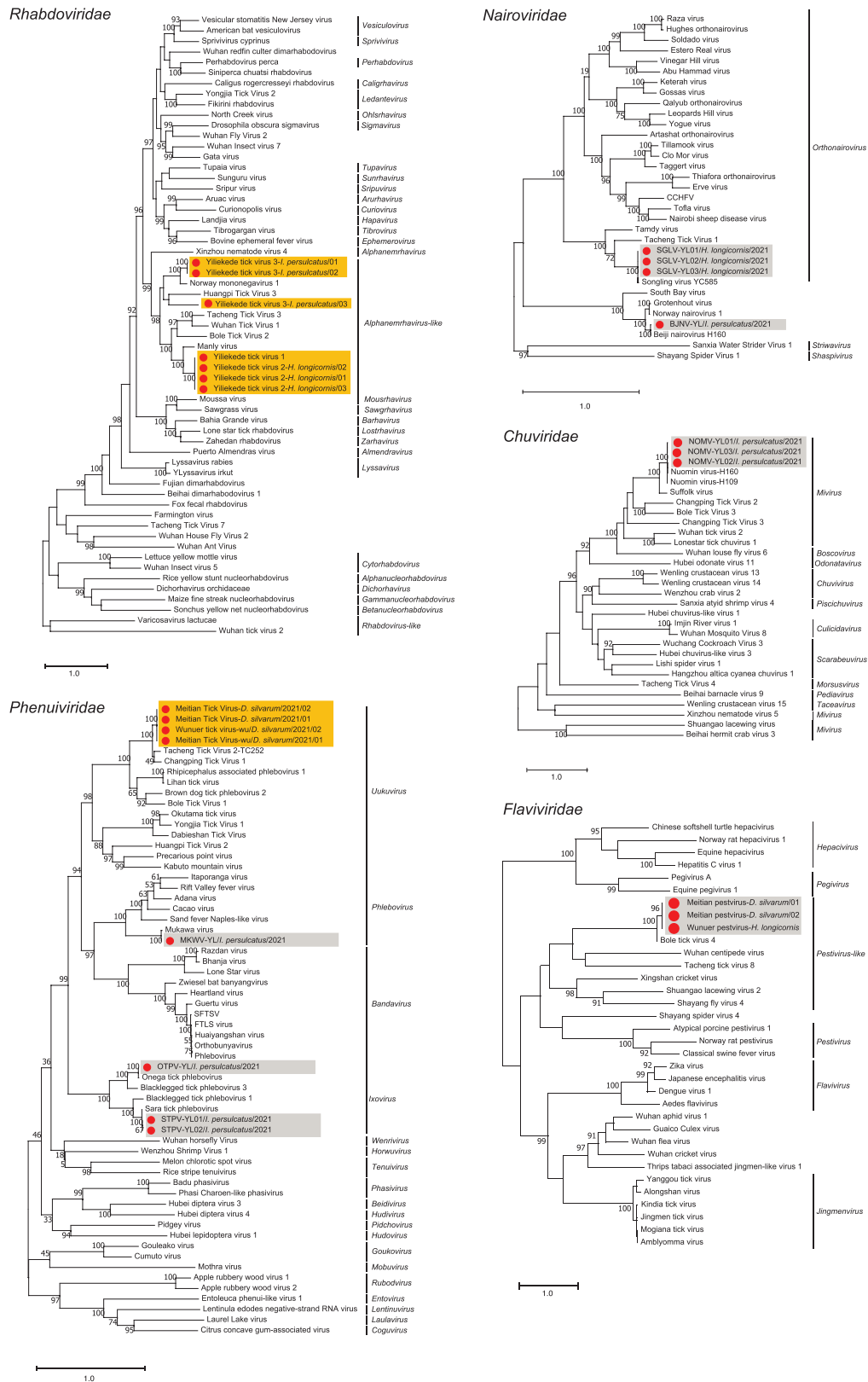


**FIGURE 3 |** Phylogenetic relationships and genomic structure of *Flaviviridae*, *Phenuiviridae*, *Chuviridae*, *Rhabdoviridae*, and *Nairoviridae* identified in this study.

A phylogenetic tree was constructed with the RdRp (*Phenuiviridae*, *Rhabdoviridae*, *Nairoviridae*, and *Chuviridae*) and NS5 (*Flaviviridae*) protein sequences of novel viruses or new viral strains identified from tick libraries with representative viruses. Novel viruses and new viral strains are marked with yellow and gray shading, respectively.

### Phenuiviridae

Our study identified a novel phlebovirus, Wunuer tick virus (WnTV), and novel variants of Onega tick phlebovirus (OTPV), Sara tick phlebovirus (STPV), and Mukawa virus (MKWV), along with a new Uukuvirus, MtTV, in *D. silvarum* ticks. The L segment of WnTV was closely related to the Changping tick virus 1 (CPTV1) identified in *Dermacentor* spp. from China, with an aa identity of 83.18% to RdRp [19]. Phylogenetic analysis of RdRp demonstrated that WnTV clustered with other CPTV-1 strains and formed a clade distant from other tick-borne phenuiviruses. OTPV-YL, STPV-YL, and MKWV-YL identified in *I. persulcatus* from Yiliegede, showed the closest relationship to OTPV Rus/*Ix\_persulcatus*/Karelia/3/2018, STPV Rus/*Ix\_persulcatus*/Karelia/4/2018, and MKW73 (97.32–98.20% to NP, 97.32–99.58% to RdRp), respectively (Fig 3). These viruses have recently been identified in *I. persulcatus* from Russia and Japan [31]. Four S and L segments of Uukuvirus-like virus MtTV in *D. silvarum* from Meitian and Wunuer shared high similarity to TcTV2 (83.01–83.38% to RdRp, 74.7–78.85% to NP), which has recently been reported in infection patients with *D. marginatus* exposure in China and Turkey [13]. Multiple alignments of MtTV from different collections showed nt identities of 88.67–95.78% in the S segment and 95.20–98.94% in the L segment.

### Nairoviridae

The Nairovirus genus belongs to the family *Nairoviridae*, which includes several important highly pathogenic TBVs such as CCHFV, Nairobi sheep disease viruses, SGLV, and BJNV. We identified three SGLV strains from *H. longicornis* ticks from Yiliegede, which were closely associated with the SGLV strain YC585 discovered in *H. longicornis* ticks collected from Heilongjiang, China, with high similarities of 95.89% (L), 92.18% (M), and 99.04% (S). Moreover, we identified a complete S segment (2,643 nt) and L segment (14,811 nt) of BJNV-YL in *I. persulcatus* from Yiliegede, near Yakeshi, where BJNV was initially identified in febrile patients [14]. The L and S segments of BJNV-YL contained a 14,459 nt and 1,659 nt ORF encoding a 4,820 aa RdRp and 552 aa NP, respectively. The BJNV-YL strain identified from Yiliegede showed high similarity to BJNV found in febrile patients (98.67% [RdRp] and 99.09% [NP] identity). Phylogenetic analysis indicated that BJNV-YL grouped into nairo-like virus clades, together with Gakugsa tick virus, South Bay virus, Norway nairovirus 1, and Grotenhout virus from Russia, the United States, Norway, and Belgium (Fig 3) [20,21].

### Rhabdoviridae

The *Rhabdoviridae* family, known for its wide host range, has a typical genome organization encoding five proteins: NP, P, M, G, and L [32]. We identified a high proportion (82.76%) of virus-related reads belonging to the *Rhabdoviridae* family from the tick virome, which were determined to represent three novel viruses: Yiliegede

tick virus 1 (YITV1) from *H. longicornis*, and Yiliegede tick virus 2 (YITV2) and Yiliegede tick virus 3 (YITV3) from *I. persulcatus* (Table 2 and Fig 3). YITV1 encoded four putative proteins and showed a distant relationship with the Nayun tick rhabdovirus NY-13, with low sequence identities (34.97% to NP, 34.87% to matrix protein (M), 46.49% to GP, and 70.01% to RdRp). Meanwhile, three viral strains of YITV3, identified as unclassified *Rhabdoviridae*-like viruses, also contained four ORFs for NP, M, GP, and RdRp, and showed the closest similarity to Norway mononegavirus 1. Among them, the aa sequences of YITV3-*I. persulcatus*/01 and YITV3-*I. persulcatus*/02 were similar to Norway mononegavirus 1 NOR/H3/Skanevik/2014 (60.65% and 61.64% to NP; 71.88% and 72.5% to M; 70.99% and 71.23% to GP; and 79.33% and 80.91% to RdRp) [33], whereas YITV3-YITV3-*I. persulcatus*/03 showed a relatively distant relationship with NOR/H3/Skanevik/2014 (42.68% to NP, 33.71% to M, 47.78% to GP, and 49.46% to RdRp). Furthermore, the three viral strains of YITV2 encoded only three proteins, NP, GP, and RdRp, which showed low sequence identities to Manly virus (34.11–34.97% to NP, 46.49% to GP, and 69.96–70.87% to RdRp). Phylogenetic analyses also demonstrated that both YITV2 and Manly virus constituted a discrete clade within an unclassified clade, provisionally designated the Alphanemrhavirus-like group. Notably, the difference in encoded protein and sequence divergence (34.11–34.97% to NP, 46.49% to GP, and 69.96–70.87% to RdRp) between YITV1 and YITV2, which were identified from the same tick species and location, indicated high diversity of mononegavirus-like viruses in Yiliegede.

### Prevalence of SGLV, BJNV, YLTV1, MtTV, and NOMV among tick populations in Inner Mongolia

To investigate the prevalence of novel viruses (MtTV and YLTV1) and new viral strains associated with febrile illnesses (NOMV, BJNV, and SGLV) identified from tick virome analysis in Hulunbuir, we performed viral detection among all 500 ticks (340 *D. silvarum*, 80 *I. persulcatus*, and 80 *H. longicornis*) collected from Meitian, Wunuer, Yiliegede, Mianduhe, Dayan, Moguai, Muyuan, Tenihe, Yakeshi, and Zhaluomude. All ticks were grouped into 32 pools (Table 3 and Fig 4). Consequently, the overall MIR was 6.4% (32/500; 95% CI: 4.41–8.91). The highest MIR was 4.4% for MtTV (22/500; 95% CI: 2.77–6.58), which was followed by NOMV (1.0%; 5/500; 95% CI: 0.32–2.32), YLTV1 (0.8%; 4/500; 95% CI: 0.21–2.03), SGLV (0.6%; 3/500; 95% CI: 0.12–1.74), and BJNV (0.4%; 2/500; 95% CI: 0.04–1.4). Of these five viruses, MtTV was found in all *D. silvarum* ticks from all collections in Hulunbuir except for Yiliegede; the MIR ranged from 8.0% in Meitian (95% CI: 2.22–19.23) to 4.0% in Wunuer (95% CI: 0.49–13.7). The presence of YLTV1 was detected in *D. silvarum* and *H. longicornis*, with MIR infection rates of 4.0% in Wunuer and 2.5% in Yiliegede. Moreover, NOMV, BJNV, and SGLV were also detected only in

**TABLE 2** | Novel viruses and viral strains identified in this study.

| Events | Family        | Virus name                                         | Novel virus | Collection  | Tick species          | Closest relative virus     | Length (nt) | Gene        | AA identity (%) |
|--------|---------------|----------------------------------------------------|-------------|-------------|-----------------------|----------------------------|-------------|-------------|-----------------|
| 1      | Chuviridae    | NOMV-YL01/ <i>I. persulcatus</i> /2021             | N           | Yiliekedede | <i>I. persulcatus</i> | Nuomin virus H160          | 10,874      | NP          | 99.5            |
|        |               |                                                    |             |             |                       |                            |             | GP          | 99.7            |
|        |               |                                                    |             |             |                       |                            |             | RdRp        | 99.6            |
|        |               | NOMV-YL02/ <i>I. persulcatus</i> /2021             |             | Yiliekedede | <i>I. persulcatus</i> | 11,337                     | NP          | 99.5        |                 |
|        |               |                                                    |             |             |                       |                            | GP          | 99.5        |                 |
|        |               |                                                    |             |             |                       |                            | RdRp        | 99.5        |                 |
|        |               | NOMV-YL03/ <i>I. persulcatus</i> /2021             |             | Yiliekedede | <i>I. persulcatus</i> | 11,321                     | NP          | 99.7        |                 |
|        |               |                                                    |             |             |                       |                            | GP          | 99.7        |                 |
|        |               |                                                    |             |             |                       |                            | RdRp        | 99.7        |                 |
| 2      | Flaviviridae  | Meitian pestivirus- <i>D. silvarum</i> /01         | Y           | Meitian     | <i>D. silvarum</i>    | Bole tick virus 4          | 16,493      | Polyprotein | 87.09           |
|        |               | Meitian pestivirus- <i>D. silvarum</i> /02         |             | Meitian     | <i>D. silvarum</i>    |                            | 16,499      | Polyprotein | 86.8            |
|        |               | Meitian pestivirus- <i>H. longicornis</i>          |             | Wunuer      | <i>H. longicornis</i> |                            | 13,593      | Polyprotein | 86.62           |
|        |               |                                                    |             |             |                       |                            |             |             |                 |
| 3      | Nairoviridae  | SGLV-YL01/ <i>H. longicornis</i> /2021             | N           | Yiliekedede | <i>H. longicornis</i> | Songling virus             | 1,767       | NP          | 99.7            |
|        |               |                                                    |             |             |                       |                            | 4,527       | GP          | 99.1            |
|        |               |                                                    |             |             |                       |                            | 12,023      | RdRp        | 99.2            |
|        |               | SGLV-YL02/ <i>H. longicornis</i> /2021             |             | Yiliekedede | <i>H. longicornis</i> | 1,808                      | NP          | 99.7        |                 |
|        |               |                                                    |             |             |                       |                            | 4,588       | GP          | 99.1            |
|        |               |                                                    |             |             |                       |                            | 12,029      | RdRp        | 99.2            |
|        |               | SGLV-YL03/ <i>H. longicornis</i> /2021             |             | Yiliekedede | <i>H. longicornis</i> | 2,417                      | NP          | 99.7        |                 |
|        |               |                                                    |             |             |                       |                            | 4,526       | GP          | 99.1            |
|        |               |                                                    |             |             |                       |                            | 12,570      | RdRp        | 99.2            |
| 4      | Nairoviridae  | BJNV-YL/ <i>I. persulcatus</i> /2021               | N           | Yiliekedede | <i>I. persulcatus</i> | Beiji nariovirus           | 14,811      | RdRp        | 98.6            |
|        |               |                                                    |             |             |                       |                            | 2,643       | NP          | 99.0            |
| 5      | Phenuiviridae | Wunuer tick virus                                  | Y           | Wunuer      | <i>H. longicornis</i> | Changping tick virus 1     | 6,640       | RdRp        | 83.1            |
| 6      | Phenuiviridae | STPV-YL01/ <i>I. persulcatus</i> /2021             | N           | Yiliekedede | <i>I. persulcatus</i> | Sara tick phlebovirus      | 1,912       | NP          | 98.3            |
|        |               |                                                    |             |             |                       |                            | 6,713       | RdRp        | 99.2            |
|        |               | STPV-YL02/ <i>I. persulcatus</i> /2021             | N           | Yiliekedede | <i>I. persulcatus</i> |                            | 2,643       | NP          | 99.2            |
|        |               |                                                    |             |             |                       | 4,758                      | RdRp        | 99.5        |                 |
| 7      | Phenuiviridae | MKWV-YL/ <i>I. persulcatus</i> /2021               | N           | Yiliekedede | <i>I. persulcatus</i> | Mukawa virus               | 1,900       | NP          | 93.0            |
|        |               |                                                    |             |             |                       |                            | 3,308       | GP          | 98.7            |
|        |               |                                                    |             |             |                       |                            | 6,425       | RdRp        | 99.0            |
| 8      | Phenuiviridae | OTPV-YL/ <i>I. persulcatus</i> /2021               | N           | Yiliekedede | <i>I. persulcatus</i> | Onega tick phlebovirus     | 1,923       | NP          | 97.7            |
|        |               |                                                    |             |             |                       |                            | 6,689       | RdRp        | 99.5            |
| 9      | Phenuiviridae | Meitian tick virus- <i>D. silvarum</i> /2021/01    | Y           | Meitian     | <i>D. silvarum</i>    | Tacheng tick virus 2-TC252 | 1,943       | NP          | 75.2            |
|        |               |                                                    |             |             |                       |                            | 6,640       | RdRp        | 83.0            |
|        |               | Meitian tick virus- <i>D. silvarum</i> /2021/02    |             | Meitian     | <i>D. silvarum</i>    |                            | 1,403       | NP          | 78.8            |
|        |               |                                                    |             |             |                       | 6,630                      | RdRp        | 83.0        |                 |
|        |               | Meitian tick virus-wu/ <i>D. silvarum</i> /2021/01 |             | Wunuer      | <i>D. silvarum</i>    |                            | 1,903       | NP          | 74.7            |
|        |               |                                                    |             |             |                       | 6,644                      | RdRp        | 83.38       |                 |

TABLE 2 | (Continued)

| Events | Family               | Virus name                                         | Novel virus | Collection | Tick species          | Closest relative virus                      | Length (nt) | Gene                       | AA identity (%)                  |
|--------|----------------------|----------------------------------------------------|-------------|------------|-----------------------|---------------------------------------------|-------------|----------------------------|----------------------------------|
|        |                      | Meitian tick virus-wu/ <i>D. silvarum</i> /2021/02 |             | Wunuer     | <i>D. silvarum</i>    |                                             | 2,042       | NP                         | 75.5                             |
| 10     | <i>Rhabdoviridae</i> | Yiliegede tick virus 1                             | Y           | Yiliegede  | <i>H. longicornis</i> | Nayun tick rhabdovirus NY-13                | 11,342      | NP<br>Matrix<br>GP<br>RdRp | 34.97<br>34.87<br>46.49<br>70.01 |
| 11     | <i>Rhabdoviridae</i> | Yiliegede tick virus 2- <i>H. longicornis</i> /01  | Y           | Yiliegede  | <i>H. longicornis</i> | Manly virus                                 | 11,349      | NP<br>GP<br>RdRp           | 34.11<br>46.49<br>69.96          |
|        |                      | Yiliegede tick virus 2- <i>H. longicornis</i> /02  | Y           | Yiliegede  | <i>H. longicornis</i> |                                             | 11,343      | NP<br>GP<br>RdRp           | 34.11<br>46.49<br>69.96          |
|        |                      | Yiliegede tick virus 2- <i>H. longicornis</i> /03  | Y           | Yiliegede  | <i>H. longicornis</i> |                                             | 10,562      | NP<br>GP<br>RdRp           | 34.97<br>46.49<br>70.78          |
| 12     | <i>Rhabdoviridae</i> | Yiliegede tick virus 3- <i>I. persulcatus</i> /01  | Y           | Yiliegede  | <i>I. persulcatus</i> | Norway mononegavirus 1 NOR/H3/Skanevik/2014 | 11,486      | NP<br>Matrix<br>GP<br>RdRp | 61.64<br>71.88<br>70.99<br>79.33 |
|        |                      | Yiliegede tick virus 3- <i>I. persulcatus</i> /02  | Y           | Yiliegede  | <i>I. persulcatus</i> |                                             | 11,488      | NP<br>Matrix<br>GP<br>RdRp | 60.65<br>72.5<br>71.23<br>80.91  |
|        |                      | Yiliegede tick virus 3- <i>I. persulcatus</i> /03  | Y           | Yiliegede  | <i>I. persulcatus</i> |                                             | 10,331      | NP<br>Matrix<br>GP<br>RdRp | 42.68<br>33.71<br>47.78<br>49.46 |

ticks from Yiliegede. MONV was detected in *I. persulcatus*, with a MIR of 3.75% (3/80; 95% CI: 0.78–10.57), and in *H. longicornis*, with a MIR of 2.50% (2/80; 95% CI: 0.30–8.74). BJNV and SGLV were observed only in *I. persulcatus* and *H. longicornis*, with detection rates of 2.5% (2/80) and 3.75% (3/80), respectively.

## DISCUSSION

Recently, many new TBVs associated with human diseases, such as ALSV [10], SGLV [11], BJNV [14], and WELV [12] have been identified in Inner Mongolia, thus highlighting the public health significance of TBVs in

Inner Mongolia. However, understanding of the distribution and reservoir host characteristics of these newly identified viruses is limited. Moreover, the diversity of TBVs in Inner Mongolia remains to be determined. Herein, we presented a metagenomic description of the viruses harbored by *D. silvarum*, *I. persulcatus*, and *H. longicornis* in Hulunbuir, Inner Mongolia, China. At least 26 viral species in nine families were identified in this study, including seven novel viruses (MtTV and YLTV1) and five new viral strains of human febrile disease-related TBVs (SGLV, BJNV, and NOMV), thereby revealing high viral diversity among ticks in Inner Mongolia. Virome comparison analysis indicated that tick species, but not tick sex, plays

**TABLE 3** | Prevalence of SGLV, BJNV, YLTV1, MtTV, and NOMV among tick populations in Inner Mongolia.

| Collections | Tick species   | Nested PCR data                      | MtTV                        | YLTV1                       | NOMV       | BJNV      | SGLV       |
|-------------|----------------|--------------------------------------|-----------------------------|-----------------------------|------------|-----------|------------|
| Meitian     | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 50/4/4<br>8.0<br>2.22–19.23 | 50/4                        | 50/4       | 50/4      | 50/4       |
| Wunuer      | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 50/4/2<br>4.0<br>0.48–13.71 | 50/4/2<br>4.0<br>0.48–13.71 | 50/4       | 50/4      | 50/4       |
| Dayan       | <i>D. sil</i>  | Numbers/pools/+<br>MIR<br>95% CI     | 30/2/2<br>6.6<br>0.08–22.07 | 30/2                        | 30/2       | 30/2      | 30/2       |
| Mianduhe    | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 30/2/2<br>6.6<br>0.08–22.07 | 30/2                        | 30/2       | 30/2      | 30/2       |
| Moguai      | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 60/4/4<br>6.6<br>0.08–22.07 | 60/6                        | 60/6       | 60/6      | 60/6       |
| Muyuan      | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 30/2/2<br>6.6<br>0.08–22.07 | 30/2                        | 30/2       | 30/2      | 30/2       |
| Tenihe      | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 30/2/2<br>6.6<br>0.08–22.07 | 30/2                        | 30/2       | 30/2      | 30/2       |
| Yakeshi     | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 30/2/2<br>6.6<br>0.08–22.07 | 30/2                        | 30/2       | 30/2      | 30/2       |
| Zhaluomude  | <i>D. sil</i>  | Numbers/pools/+<br>MIR (%)<br>95% CI | 30/2/2<br>6.6<br>0.08–22.07 | 30/2                        | 30/2       | 30/2      | 30/2       |
| Yiliegede   | <i>I. pers</i> | Numbers/pools/+                      | 80/4                        | 80/4                        | 80/4/3     | 80/4/2    | 80/4       |
|             |                | MIR (%)                              |                             |                             | 3.75       | 2.5       |            |
|             |                | 95% CI                               |                             |                             | 0.78–10.57 | 0.30–8.74 |            |
|             | <i>H. long</i> | Numbers/pools/+                      | 80/4                        | 80/4/2                      | 80/4/2     | 80/4      | 80/4/3     |
|             |                | MIR (%)                              |                             | 2.5                         | 2.5        |           | 3.75       |
|             |                | 95% CI                               |                             | 0.30–8.74                   | 0.30–8.74  |           | 0.78–10.57 |
| Subtotal    |                | Numbers/pools/+                      | 500/32/22                   | 500/32/4                    | 500/32/5   | 500/32/2  | 500/32/3   |
|             |                | MIR (%)                              | 4.4                         | 0.8                         | 1          | 0.4       | 0.6        |
|             |                | 95% CI                               | 2.77–6.58                   | 0.21–2.03                   | 0.325–2.32 | 0.04–1.4  | 0.12–1.74  |

*I. pers*, *I. persulcatus*; *D. sil*, *D. silvarum*; *H. long*, *H. longicornis*; +, positive samples; MIR, minimum infection rate (%); CI, confidence interval.

an important role in shaping the viral spectrum carried by ticks in Hulunbuir. Furthermore, in agreement with the virome distribution findings, the surveillance of SGLV,

BJNV, MtTV, and NOMV among tick groups indicated that MtTV identified from *D. silvarum* was detected in *D. silvarum* ticks from the nine counties with the highest MIR

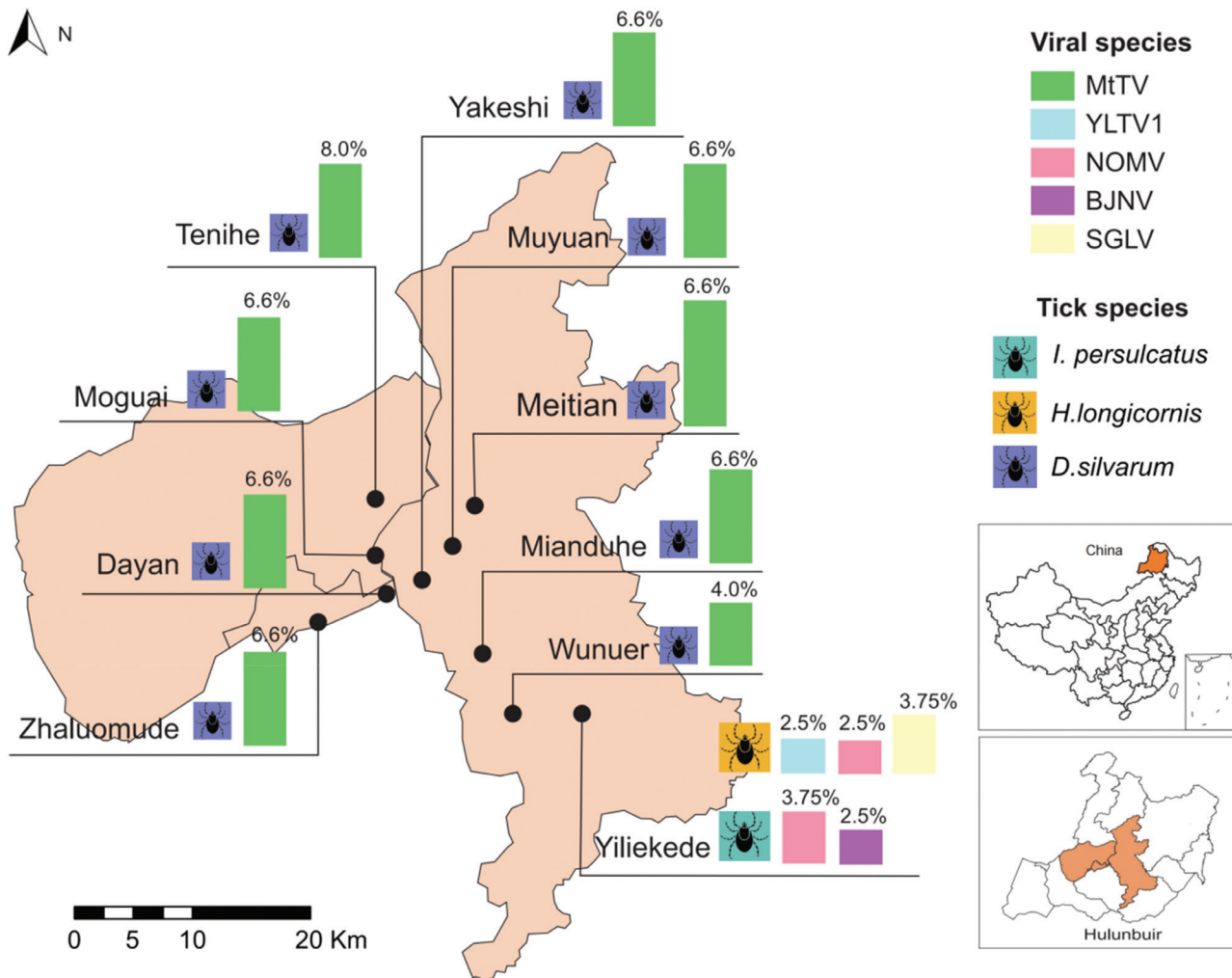


**FIGURE 4** | Overview of the ecological distribution characteristics of MtTV, YLTV1, NOMV, BJNV, and SGLV found in Hulunbuir, based on tick group screening results. The minimum infection rate of each virus is shown in bar plots. The detected tick species are indicated by icons as follows: blue for *I. persulcatus*, yellow for *H. longicornis*, and purple for *D. silvarum*.

of 4–8%. These findings suggested a wide distribution of this novel MtTV Uukuvirus among *D. silvarum* ticks in Hulunbuir. SGLV, BJNV, and NOMV identified from *I. persulcatus* and *H. longicornis* tick libraries in Yiliegede were also detected in corresponding tick species in Yiliegede, with MIR values of 0.6%, 0.4%, and 1.0%, respectively; these findings indicated the potential spillover risk of these infectious viruses in Yiliegede. Our study expands understanding of the geographic distribution and phylogenetic diversity of novel TBVs in Inner Mongolia, and highlights their medical and public health importance.

Our sampling results regarding tick species distribution were similar to the outcomes reported throughout Mongolia [34], Russia [35], and Northern Europe [33] at similar latitudes: *Dermacentor* spp., *Ixodes* spp., and *Haemaphysalis* spp. were the most abundant tick species in this region. These three tick species play important roles in the spread of TBVs worldwide. We observed distinct virome characteristics among *D. silvarum*, *I. persulcatus*, and *H. longicornis* ticks in Inner Mongolia. The *I. persulcatus*

tick libraries exhibited the highest number and proportion of viral reads, in agreement with previous findings that *I. persulcatus* is a significant vector for various pathogens, because of its wide host range and adaptability. In contrast, *H. longicornis* and *D. silvarum* ticks carry a diverse virome, and showed lower viral load and species richness than *I. persulcatus*. At the viral family level, *Flaviviridae* and *Phenuiviridae* were more frequently detected in *D. silvarum*, whereas *Chuviridae* predominated in *I. persulcatus*, and *Narioviridae* predominated in *H. longicornis*. This distribution reflected each tick species' unique ecological niches and host interactions influencing virome composition. Alpha diversity analysis revealed higher Shannon indices in *D. silvarum* than *I. persulcatus*, thus suggesting greater viral diversity in the former. The beta diversity analysis showed significant differences in viral species composition among the tick species, and highlighted the importance of species-specific factors in shaping the virome. Furthermore, our findings indicated that tick species plays a crucial role in determining the spectrum of carried

viruses in Inner Mongolia. The virome patterns from the same tick species clustered separately, with *I. persulcatus* ticks predominantly harboring NOMV and other specific viruses; *H. longicornis* ticks showing a high abundance of SGLV and Wuhan insect virus 7; and *D. silvarum* ticks uniquely carrying bole tick virus 4 and Tacheng tick virus 2. Interestingly, viral distribution and abundance showed no significant differences between tick sex or locations. These findings suggested that species-specific factors outweigh these variables in influencing virome composition. The observed geographic invasion has drawn attention to the *H. longicornis* population and its associated emerging disease threats. In China, viruses belonging to the families *Nairoviridae* and *Phenuiviridae* in the order *Bunyavirales* are most commonly detected with high abundance and prevalence among *H. longicornis* [36,37]. Almost all pools of *H. longicornis* presented viral sequences, including the nairo-like virus SGLV, which was first isolated from Inner Mongolia and detected in *Ixodes* spp. and *Haemaphysalis* spp. [11]. These results expand knowledge of the diversity of viruses carried by these three tick species in Inner Mongolia and provide guidance for the future surveillance of viruses carried by these ticks.

Chuviruses, first identified in 2015 through metagenomics in various arthropods, have been classified into the family *Chuviridae* within the order *Jingchuvirales*, because of their diverse genomic structures, including non-segmented, segmented, and circular forms [38]. Recent studies have indicated the widespread presence of chuviruses across various regions, including China [19], Kenya [23], Thailand [39], the United States [40], and Australia [41], thus underscoring their ecological and public health significance. NOMV, a novel chuvirus, was initially identified in patients with febrile illness in northeastern China. Between 2017 and 2019, 34 NOMV human infection cases were reported in Inner Mongolia, and NOMV was also detected in *I. persulcatus* in the Alongshan region, thereby suggesting a potential risk of spillover and transmission in Inner Mongolia. Our identification of the presence of NOMV in *I. persulcatus* and *H. longicornis* ticks in the Yiliekedede region of Inner Mongolia extends understanding of NOMV's prevalence and vector diversity in Inner Mongolia. Additionally, we observed NOMV prevalence rates of 3.75% in *I. persulcatus* and 2.0% in *H. longicornis*, values similar to the positivity rate range of 1.9–8.2% observed in ticks from northeastern China. Because of the MIR calculation used in our study, the substantial infection rates in Inner Mongolian ticks might potentially exceed the observed MIR values of 3.75% and 2.5%. Furthermore, different tick species from different regions might have distinct infection rates because of their unique ecological niches and host interactions. NOMV is influenced by regional factors such as climate, vegetation, and host availability, which can affect tick populations and the transmission dynamics of the virus. Our findings emphasize the need for diligent monitoring of the public

health effects of NOMV in Inner Mongolia to effectively address the emerging challenges posed by this pathogen.

Most known members of the family *Flaviviridae* are transmitted by arthropods, and several are important zoonotic agents, which have been reported in Inner Mongolia, Heilongjiang, Jilin, and Xinjiang in China [42]. We identified three novel viruses belonging to the *Flaviviridae* family that were most similar to the pest-like virus BLTV4, which has been recently identified in China [19], Thailand [39], and Kenya [23]. These findings contribute to the growing body of evidence highlighting the diverse flavivirus carried by ticks. Moreover, the high similarity of pest-like virus BLTV4 variants between Meitian and Wunuer in Inner Mongolia (98.3%) indicates high conservation of this novel flavivirus among ticks in Hulunbuir. This conservation might facilitate the spread of these viruses across geographical areas, and consequently pose risks to both animal and human health.

An increasing number of emerging viruses belonging to the family *Phenuiviridae* have been identified within various tick species and are classified into several genera, including Bandavirus, Phlebovirus, and Uukuvirus [43]. We observed high genetic divergence of novel viruses and new viral strains viruses belonging to the family *Phenuiviridae*; the viruses included WnTV, STPV-YL, MKWV-YL, OTPV-YL, and MtTV, which formed a separate cluster within the tick-borne phlebovirus group. OTPV and STPV exhibit high genomic similarity to strains found in the Karelia region of Russia [44]. Therefore, these viruses maintain high genetic consistency within their respective populations. However, their significant genetic differences with respect to other known tick-borne phleboviruses suggest unique evolutionary paths and potential variations in their biological characteristics. MKWV, initially discovered in Japan and later detected in *Ixodes persulcatus* and *Haemaphysalis concinna* ticks in Northeast China, has been shown to infect Vero cells and consequently to replicate in mammalian cells [45]. This finding prompts concerns regarding its potential for zoonotic transmission, given its widespread distribution in Northeast China. Therefore, further testing of a broader range of tick species, as well as wild animals, domestic animals, and humans, is of practical importance to assess the public health implications. Additionally, MtTV was detected exclusively in *D. silvarum*, and exhibited the highest MIR and the broadest distribution among tested viruses. The presence of MtTV in all *D. silvarum* groups from nine sampling locations strongly suggested stable and widespread circulation of this virus among *D. silvarum* in Inner Mongolia. Although no current evidence indicates the pathogenicity of MtTV, the closest phylogenetic relationship of this virus was with Tacheng tick virus 2 (75.2–83.38%, aa identity), which is associated with human febrile illnesses. Therefore, the potential public health implications of MtTV warrant further investigation.

The genus *Orthonairovirus*, belonging to the family *Nairoviridae*, contains numerous highly pathogenic zoonotic

TBVs, such as CCHFV and the Nairobi sheep disease virus, causing fatal hemorrhagic fever and hemorrhagic gastroenteritis in humans as well as animals in Asia, Africa, and Europe [46]. Recently, several newly discovered viruses have been identified in ticks and mammals. SGLV was first isolated from patients who reported being bitten by ticks in Heilongjiang Province in 2021 [11]. Subsequently, another nairo-like virus, BJNV, was identified in patients from Inner Mongolia [14]. Here, we reported the presence of two viral strains from Yiliekedede showing high similarity (98–99%) to SGLV and BJNV identified from *H. longicornis* and *I. persulcatus* pools, respectively. Furthermore, SGLV and BJNV were detected with nested RT-PCR in tick groups. A previous study has indicated that SGLV was prevalent in four hard ticks in Heilongjiang, and *I. crenulatus* might be the main tick species vector for SGLV (with a prevalence of 5.5%) [11]. However, in Yiliekedede, SGLV was detected only in *H. longicornis* (MIR: 3.75%) in our study, probably because of the limited numbers and species of ticks collected. Moreover, the positivity rate of BJNV measured in *I. persulcatus* (MIR: 2.5%) was similar to findings from a survey of BJNV prevalence in ticks (0.4–2.1%) in hilly and wooded regions of Inner Mongolia [14]. Previous reports and our current study demonstrate the circulation of these infectious nairoviruses in Inner Mongolia and indicates that the potential serological exposure to SGLV and BJNV among humans and animals in Yiliekedede warrants further validation.

*Rhabdoviridae*, a group of single-stranded negative-sense RNA viruses, infect animals and plants, and their frequent host switching during evolution has led to considerable variability in viral sequences among viral members [32]. Herein, a distinct and divergent spectrum of novel Rhabdoviruses (YLTV1, YLTV2, and YLTV3) was discovered in Yiliekedede and found to have low aa similarity with the Nayun tick rhabdovirus NY-13 first identified in China [47], Manly virus in Australia [41], and Norway mononegavirus 1 [33], thus indicating that substantial diversity of rhabdoviruses has not yet been sampled. Closer examination of the genomic structures of YLTV1, YLTV2, and YLTV3 indicated distinct differences. Both YLTV1 and YLTV3 encode four proteins, whereas YLTV2 encodes only three, omitting the M protein. This variation in genomic composition is poised to influence virion assembly, morphology, and functionality. The M protein is instrumental in preserving the structural integrity and morphological attributes of virions in many viruses. Thus, the absence of the M protein in YLTV2 is likely to confer unique morphological characteristics or infective properties to virions. Moreover, a comparative analysis of YLTV1 and NY-13 revealed substantial differences in genomic coding proteins and phylogenetic relationships. Notably, the aa similarity of the NP and M proteins below 35% suggested significant evolutionary distance between YLTV1 and NY-13 in terms of the genomic sequence and coding proteins. This divergence might indicate that YLTV1 is a novel branch or species within the *Rhabdoviridae* family. The low similarity suggests that

YLTV1 might have unique biological characteristics, such as distinct host adaptability or infection mechanisms; this possibly requires further validation through experimental research. In addition, in alignment with the tick virome findings, we detected the presence of NYTRV in *I. persulcatus* and *D. silvarum* ticks from Yiliekedede and Wunuer via nested PCR, which indicated a wide distribution of NYTRV within the Hulunbuir region.

This research shed light on the variety and distribution of TBVs in Hulunbuir, Inner Mongolia, and uncovered several novel viruses and new strains associated with known infectious TBVs, such as SGLV and NOMV. Surveillance across three dominant tick species at ten collection sites in Hulunbuir also revealed varying infection rates of SGLV, BJNV, YLTV1, MtTV, and NOMV. These findings underscore not only the widespread distribution of TBVs in Hulunbuir but also the critical need for region-specific strategies in monitoring and controlling TBVD. Our study highlights the potential circulation of emergent TBVs between ticks and their reservoir hosts, and indicates a significant public health risk amplified by inter-urban interactions. Finally, our findings substantially enrich understanding of TBVs, and offer crucial insights for the prevention and control of these viruses and their associated diseases in northern China.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from [https://zoonoses-journal.org/wp-content/uploads/2025/03/zoonoses20240064\\_Suppl.pdf](https://zoonoses-journal.org/wp-content/uploads/2025/03/zoonoses20240064_Suppl.pdf).

## REFERENCES

1. Pielnaa P, Al-Saadawe M, Saro A, Dama MF, Zhou M, Huang Y, et al. Zika virus-spread, epidemiology, genome, transmission cycle, clinical manifestation, associated challenges, vaccine and antiviral drug development. *Virology*. 2020;543:34-42.
2. Kularatne SA, Dalugama C. Dengue infection: global importance, immunopathology and management. *Clin Med (Lond)*. 2022;22(1):9-13.
3. Tuells J, Henao-Martínez AF, Franco-Paredes C. Yellow fever: a perennial threat. *Arch Med Res*. 2022;53(7):649-657.
4. Kuehnert PA, Stefan CP, Badger CV, Ricks KM. Crimean-Congo hemorrhagic fever virus (CCHFV): a silent but widespread threat. *Curr Trop Med Rep*. 2021;8(2):141-147.

5. Pulkkinen LIA, Butcher SJ, Anastasina M. Tick-borne encephalitis virus: a structural view. *Viruses*. 2018;10(7):350.
6. Li J, Li S, Yang L, Cao P, Lu J. Severe fever with thrombocytopenia syndrome virus: a highly lethal bunyavirus. *Crit Rev Microbiol*. 2021;47(1):112-125.
7. Krasteva S, Jara M, Frias-De-Diego A, Machado G. Nairobi Sheep Disease virus: a historical and epidemiological perspective. *Front Vet Sci*. 2020;7:419.
8. Gaudreault NN, Madden DW, Wilson WC, Trujillo JD, Richt JA. African swine fever virus: an emerging DNA arbovirus. *Front Vet Sci*. 2020;7:215.
9. Moming A, Bai Y, Wang J, Zhang Y, Tang S, Fan Z, et al. The known and unknown of global tick-borne viruses. *Viruses*. 2024;16(12):1807.
10. Wang ZD, Wang B, Wei F, Han SZ, Zhang L, Yang ZT, et al. A new segmented virus associated with human febrile illness in China. *N Engl J Med*. 2019;380(22):2116-2125.
11. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat Med*. 2021;27(3):434-439.
12. Zhang XA, Ma YD, Zhang YF, Hu ZY, Zhang JT, Han S, et al. A new orthonairovirus associated with human febrile illness. *N Engl J Med*. 2024;391(9):821-831.
13. Dong Z, Yang M, Wang Z, Zhao S, Xie S, Yang Y, et al. Human Tacheng tick virus 2 infection, China, 2019. *Emerg Infect Dis*. 2021;27(2):594-598.
14. Wang YC, Wei Z, Lv X, Han S, Wang Z, Fan C, et al. A new nairo-like virus associated with human febrile illness in China. *Emerg Microbes Infect*. 2021;10(1):1200-1208.
15. Wang ZD, Wang W, Wang NN, Qiu K, Zhang X, Tana G, et al. Prevalence of the emerging novel Alongshan virus infection in sheep and cattle in Inner Mongolia, northeastern China. *Parasit Vectors*. 2019;12(1):450.
16. Kong Y, Yan C, Liu D, Jiang L, Zhang G, He B, et al. Phylogenetic analysis of Crimean-Congo hemorrhagic fever virus in inner Mongolia, China. *Ticks Tick Borne Dis*. 2022;13(1):101856.
17. Li Y, Yan C, Liu D, He B, Tu C. Seroepidemiological Investigation of Crimean-Congo hemorrhagic fever virus in sheep and camels of Inner Mongolia of China. *Vector Borne Zoonotic Dis*. 2020;20(6):461-467.
18. Qin H, Xin X, Tang Q, Feng X, Yin B. The Prevalence of tick-borne encephalitis virus in the ticks and humans of China from 2000 to 2023: a systematic review and meta-analysis. *Vet Sci*. 2025;12:146.
19. Li CX, Shi M, Tian JH, Lin XD, Kang YJ, Chen LJ, et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife*. 2015;4:e05378.
20. Tokarz R, Williams SH, Sameroff S, Sanchez Leon M, Jain K, Lipkin WI. Virome analysis of *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis* ticks reveals novel highly divergent vertebrate and invertebrate viruses. *J Virol*. 2014;88(19):11480-11492.
21. Cross ST, Kapuscinski ML, Perino J, Maertens BL, Weger-Lucarelli J, Ebel GD, et al. Co-infection patterns in individual ixodes scapularis ticks reveal associations between viral, eukaryotic and bacterial microorganisms. *Viruses*. 2018;10(7):388.
22. Ma R, Li C, Tian H, Zhang Y, Feng X, Li J, et al. The current distribution of tick species in Inner Mongolia and inferring potential suitability areas for dominant tick species based on the MaxEnt model. *Parasit Vectors*. 2023;16(1):286.
23. Zhang Y, Hu B, Agwanda B, Fang Y, Wang J, Kuria S, et al. Viromes and surveys of RNA viruses in camel-derived ticks revealing transmission patterns of novel tick-borne viral pathogens in Kenya. *Emerg Microbes Infect*. 2021;10(1):1975-1987.
24. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol*. 2011;29(7):644-652.
25. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie2. *Nat Methods*. 2012;9(4):357-359.
26. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. *BMC Bioinformatics*. 2009;10:421.
27. Ni XB, Cui XM, Liu JY, Ye RZ, Wu YQ, Jiang JF, et al. Metavirome of 31 tick species provides a compendium of 1,801 RNA virus genomes. *Nat Microbiol*. 2023;8(1):162-173.
28. Wille M, Harvey E, Shi M, Gonzalez-Acuña D, Holmes EC, Hurt AC. Sustained RNA virome diversity in Antarctic penguins and their ticks. *ISME J*. 2020;14(7):1768-1782.
29. Pettersson JH, Ellström P, Ling J, Nilsson I, Bergström S, González-Acuña D, et al. Circumpolar diversification of the *Ixodes uriae* tick virome. *PLoS Pathog*. 2020;16(8):e1008759.
30. Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, et al. ICTV virus taxonomy profile: Flaviviridae. *J Gen Virol*. 2017;98(1):2-3.
31. Matsuno K, Kajihara M, Nakao R, Nao N, Mori-Kajihara A, Muramatsu M, et al. The unique phylogenetic position of a novel tick-borne phlebovirus ensures an ixodid origin of the genus Phlebovirus. *mSphere*. 2018;3(3):e00239-18.
32. Longdon B, Murray GG, Palmer WJ, Day JP, Parker DJ, Welch JJ, et al. The evolution, diversity, and host associations of rhabdoviruses. *Virus Evol*. 2015;1(1):vev014.
33. Pettersson JH, Shi M, Bohlin J, Eldholm V, Brynildsrud OB, Paulsen KM, et al. Characterizing the virome of *Ixodes ricinus* ticks from northern Europe. *Sci Rep*. 2017;7(1):10870.
34. Černý J, Buyannemekh B, Needham T, Gankhuyag G, Oyuntsetseg D. Hard ticks and tick-borne pathogens in Mongolia – a review. *Ticks Tick Borne Dis*. 2019;10(6):101268.
35. Kholodilov IS, Belova OA, Morozkin ES, Litov AG, Ivannikova AY, Makenov MT, et al. Geographical and tick-dependent distribution of flavi-like Alongshan and Yanggou tick viruses in Russia. *Viruses*. 2021;13(3):458.
36. Ma C, Zhang R, Zhou H, Yu G, Yu L, Li J, et al. Prevalence and genetic diversity of Dabieshan tick virus in Shandong Province, China. *J Infect*. 2022;85(1):90-122.
37. Ye RZ, Li YY, Xu DL, Wang BH, Wang XY, Zhang MZ, et al. Virome diversity shaped by genetic evolution and ecological landscape of *Haemaphysalis longicornis*. *Microbiome*. 2024;12(1):35.
38. Di Paola N, Dheilly NM, Junglen S, Paraskevopoulou S, Postler TS, Shi M, et al. Jingchuvirales: a new taxonomical framework for a rapidly expanding order of unusual monjiviricete viruses broadly distributed among Arthropod subphyla. *Appl Environ Microbiol*. 2022;88(6):e0195421.
39. Temmam S, Chrétien D, Bigot T, Dufour E, Petres S, Desquesnes M, et al. Monitoring silent spillovers before emergence: a pilot study at the tick/human interface in Thailand. *Front Microbiol*. 2019;10:2315.
40. Tufts DM, Sameroff S, Tagliafierro T, Jain K, Oleynik A, VanAcker MC, et al. A metagenomic examination of the pathobiome of the invasive tick species, *Haemaphysalis longicornis*, collected from a New York City borough, USA. *Ticks Tick Borne Dis*. 2020;11(6):101516.
41. Harvey E, Rose K, Eden JS, Lo N, Abeyesuriya T, Shi M, et al. Extensive diversity of RNA viruses in Australian ticks. *J Virol*. 2019;93(3):e01358-18.
42. Li X, Ji H, Wang D, Che L, Zhang L, Li L, et al. Molecular detection and phylogenetic analysis of tick-borne encephalitis virus in ticks in northeastern China. *J Med Virol*. 2022;94(2):507-513.
43. Sun MH, Ji YF, Li GH, Shao JW, Chen RX, Gong HY, et al. Highly adaptive Phenuiviridae with biomedical importance in multiple fields. *J Med Virol*. 2022;94(6):2388-2401.
44. Liu Z, Li L, Xu W, Yuan Y, Liang X, Zhang L, et al. Extensive diversity of RNA viruses in ticks revealed by

- metagenomics in northeastern China. PLoS Negl Trop Dis. 2022;16(12):e0011017.
45. Wang YN, Jiang RR, Ding H, Zhang XL, Wang N, Zhang YF, et al. First detection of Mukawa Virus in *Ixodes persulcatus* and *Haemaphysalis concinna* in China. Front Microbiol. 2022;13:791563.
46. Ergönül O. Crimean-Congo haemorrhagic fever. Lancet Infect Dis. 2006;6(4):203-214.
47. Xia H, Hu C, Zhang D, Tang S, Zhang Z, Kou Z, et al. Metagenomic profile of the viral communities in *Rhipicephalus* spp. ticks from Yunnan, China. PLoS One. 2015;10(3):e0121609.