**Supplementary Data**

**Viromes of *Haemaphysalis longicornis* reveal different viral abundance and diversity in free and engorged ticks**

Jian Xiao a, c, #, Xuan Yao b, #, Xuhua Guan b, Jinfeng Xiong b, Yaohui Fang a, Jingyuan Zhang a, You Zhang a, f, Abulimiti Moming a, e, Zhengyuan Su a, Jiayin Jin a, Yingying Ge a, Jun Wang a, Zhaojun Fan a, Shuang Tang a, Shu Shen a, d, e, \*, Fei Deng a, \*

a Key Laboratory of Virology and Biosafety and National Virus Resource Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China

b Hubei Provincial Center for Disease Control and Prevention, Wuhan, 430070, China

c University of Chinese Academy of Sciences, Beijing, 101408, China

d Hubei Jiangxia Laboratory, Wuhan, 430200, China

e Xinjiang Key Laboratory of Vector-borne Infectious Diseases, Urumqi, 830002, China

f Current address: Department of Medical laboratory, the Second Affiliated Hospital, Hainan Medical University, Haikou, 57000, China

\* Corresponding authors.

Email addresses: [shenshu@wh.iov.cn (S](mailto:shenshu@wh.iov.cn (S). Shen), [df@wh.iov.cn (F](mailto:df@wh.iov.cn (F). Deng)

ORCID: 0000-0002-0013-5365 (S. Shen); 0000-0002-5385-083X (F. Deng)

# Jian Xiao and Xuan Yao contributed equally to the manuscript.

**Supplementary tables**

**Supplementary Table S1.** Summary of total tick sample collections in Hubei Province from 2019 to 2020.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Location** | **Feeding States** | |
|  | **Free Ticks** | **Engorged Ticks** |
| 1 | Nanzhang, Xiangyang | 80 | 79 |
| 2 | Suixian, Suizhou | 216 | 60 |
| 3 | Macheng, Huanggang | 173 | 82 |
| 4 | Yingshan, Huanggang | 90 | 20 |
|  | **Total** | **559** | **241** |

**Supplementary Table S2.** The tick pools used for RNA-seq.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Pool** | **Location** | **Number of ticks** | **Data size (Gib)** | **Virus abundance  (TPM, %)** |
| **Free ticks** | | |  |  |  |
| 1 | f1 | Yingshan, Huanggang | 30 | 28 | 1020.22, 0.01 |
| 2 | f2 | Macheng, Huanggang | 22 | 14.4 | 619.96, 0.06 |
| 3 | f3 | Macheng, Huanggang | 60 | 9.3 | 28.63, 0.00286 |
| 4 | f4 | Macheng, Huanggang | 75 | 8.9 | 23.84, 0.00238 |
| 5 | f5 | Macheng, Huanggang | 9 | 10.4 | 37.62, 0.00376 |
| 6 | f6 | Suixian, Suizhou | 83 | 8.9 | 18.28, 0.00183 |
| 7 | f7 | Suixian, Suizhou | 40 | 9.9 | 21.69, 0.00217 |
| 8 | f8 | Nanzhang, Xiangyang | 40 | 15.6 | 29908.25, 2.99 |
| **Engorged ticks** | | |  |  |  |
| 9 | e1 | Yingshan, Huanggang | 50 | 16.5 | 1310.17, 0.13 |
| 10 | e2 | Macheng, Huanggang | 25 | 19.1 | 1.65, 0.00016 |
| 11 | e3 | Macheng, Huanggang | 8 | 12.2 | 722.71, 0.07 |
| 12 | e6 | Suixian, Suizhou | 28 | 7.8 | 23.37, 0.00234 |
| 13 | e7 | Suixian, Suizhou | 24 | 43 | 64.95, 0.01 |
| 14 | e8 | Nanzhang, Xiangyang | 20 | 11.9 | 84.31, 0.01 |
|  |  | **Total** | **514** | **237.9** |  |

**Supplementary Table S3.** The primers and probes specific for viruses used in beads-based assays.

|  |  |  |
| --- | --- | --- |
| **Virus** |  | **Sequences (5**′ **to 3**′**)** |
| Jingmen tick virus | F-primer | GGAATGGCTGTTGCCCTTGGT |
|  | R-primer | GACGAGGAGACGCCTGTTGC (5′Biotin) |
|  | probe | GGCCCCGATTGATAAGGGATC |
| Henan tick virus | F-primer | ACACCGTTGCAGGAGGTCCA |
|  | R-primer | CCGGGGAGAAAAGGACGCAG (5′Biotin) |
|  | probe | GGAGCACAGTCTGCCAGTCG |
| Dabieshan tick virus | F-primer | CCATGCCGAACGAGCAGGAC |
|  | R-primer | TCACGGATGGGCTTGGTCCG (5′Biotin) |
|  | probe | GCACCGGCTCTACCTGGTTG |
| Okutama tick virus | F-primer | CCCTGGACTTCCTGGACGCA |
|  | R-primer | TGCTCGAACGACTCGGCGAT (5′Biotin) |
|  | probe | TCAACCCTGACATGCGGGGC |

**Supplementary Table S4.** Primers and probes used in the quantitative PCR detection of Henan tick virus and Dabieshan tick virus.

|  |  |  |
| --- | --- | --- |
| **Virus** |  | **Sequences (5**′ **to 3**′**)** |
| Henan tick virus | F-primer | TGTCACTATGCTGTGCCCAACT |
|  | R-primer | CGCTTCCAAATCAATGATCTCA |
|  | probe | (6-FAM) TGCCTCTGAGGAGCCTGTCGTG (BHQ1) |
| Dabieshan tick virus | F-primer | GGCTACGGCAGCACTTTCA |
|  | R-primer | TGACCACCCCCAGCTTCTT |
|  | probe | (6-FAM) CGGATGAGGCCAACCGCAAG (BHQ1) |

**Supplementary Table S5.** A summary of viruses identified in tick pools by merging the RNA-seq data of the same location.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **No.** | **Virus Family** | **Virus Species** | **Macheng, Huanggang** | **Suixian, Suizhou** | **Yingshan, Huanggang** | **Nanzhang, Xiangyang** |
| 1 | *Chuviridae* | Bole Tick Virus 3 | + | + | − | − |
| 2 | *Chuviridae* | Changping Tick Virus 2 | + | + | − | − |
| 3 | *Chuviridae* | Chuviridae sp. | − | + | − | − |
| 4 | *Chuviridae* | Hebei mivirus 2 | − | + | − | − |
| 5 | *Chuviridae* | Karukera tick virus | + | − | − | − |
| 6 | *Chuviridae* | Wuhan tick virus 2 | + | − | − | + |
| 7 | *Chuviridae* | Xinjiang mivirus 1 | − | + | − | − |
| 8 | *Nairoviridae* | Huangpi Tick Virus 1 | − | + | − | − |
| 9 | *Nairoviridae* | Taggert virus | − | + | − | − |
| 10 | *Orthomyxoviridae* | Granville quaranjavirus | − | + | − | + |
| 11 | *Orthomyxoviridae* | Ohshima virus | + | + | − | − |
| 12 | *Orthomyxoviridae* | Zambezi tick virus 1 | + | − | + | + |
| 13 | *Parvoviridae* | Lone star tick densovirus 1 | + | + | − | − |
| 14 | *Phenuiviridae* | Lihan tick virus | − | − | − | + |
| 15 | *Phenuiviridae* | Dabieshan Tick Virus | + | + | + | + |
| 16 | *Phenuiviridae* | Kismayo virus | − | + | − | − |
| 17 | *Phenuiviridae* | Lone Star virus | + | − | + | − |
| 18 | *Phenuiviridae* | Okutama tick virus | − | − | − | + |
| 19 | *Phenuiviridae* | Rhipicephalus associated phlebovirus 1 | − | − | − | + |
| 20 | *Rhabdoviridae* | Huangpi Tick Virus 3 | + | + | − | − |
| 21 | *Rhabdoviridae* | IRE/CTVM19 associated rhabdovirus | + | − | − | − |
| 22 | *Rhabdoviridae* | Tacheng Tick Virus 3 | + | − | − | − |
| 23 | *Rhabdoviridae* | Wuhan Tick Virus 1 | + | − | − | − |
| 24 | *Totiviridae* | Lonestar tick totivirus | + | + | − | − |
| 25 | *Totiviridae* | Xinjiang tick totivirus 2 | + | + | − | − |
| 26 | unclassified viruses | Alongshan virus | + | + | − | − |
| 27 | unclassified viruses | Jingmen tick virus | + | + | + | + |
| 28 | unclassified viruses | Bole tick virus 4 | + | + | − | − |
| 29 | unclassified viruses | Henan tick virus | − | + | − | − |
| 30 | unclassified viruses | Hepelivirales sp. | + | + | + | − |
| 31 | unclassified viruses | Hubei sobemo like virus 15 | + | + | − | − |
| 32 | unclassified viruses | Hubei toti like virus 24 | + | + | − | − |
| 33 | unclassified viruses | Ixodes scapularis associated virus 2 | + | − | + | − |
| 34 | unclassified viruses | Liman tick virus | + | + | − | − |
| 35 | unclassified viruses | Lone star tick associated virus-1 | − | − | + | + |
| 36 | unclassified viruses | Manly virus | + | + | − | − |
| 37 | unclassified viruses | Norway mononegavirus 1 | + | − | − | − |
| 38 | unclassified viruses | Shanxi tick virus 1 | − | + | − | − |
| 39 | unclassified viruses | Tick borne tetravirus like virus | + | + | − | − |
|  |  | **Total** | **26** | **26** | **7** | **9** |

**Supplementary Table S6.** A summary of viruses in the free and engorged ticks by integrating the data of RNA-seq from the pools.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No.** | **Virus Family** | **Virus Species** | **Free ticks** | **Engorged ticks** |
| 1 | *Chuviridae* | Bole Tick Virus 3 | + | + |
| 2 | *Chuviridae* | Changping Tick Virus 2 | + | + |
| 3 | *Orthomyxoviridae* | Granville quaranjavirus | + | + |
| 4 | *Orthomyxoviridae* | Zambezi tick virus 1 | + | + |
| 5 | *Parvoviridae* | Lone star tick densovirus 1 | + | + |
| 6 | *Phenuiviridae* | Dabieshan Tick Virus | + | + |
| 7 | *Phenuiviridae* | Kismayo virus | + | + |
| 8 | *Rhabdoviridae* | Huangpi Tick Virus 3 | + | + |
| 9 | *Totiviridae* | Lonestar tick totivirus | + | + |
| 10 | *Totiviridae* | Xinjiang tick totivirus 2 | + | + |
| 11 | unclassified viruses | Alongshan virus | + | + |
| 12 | unclassified viruses | Bole tick virus 4 | + | + |
| 13 | unclassified viruses | Hepelivirales sp. | + | + |
| 14 | unclassified viruses | Hubei toti like virus 24 | + | + |
| 15 | unclassified viruses | Ixodes scapularis associated virus 2 | + | + |
| 16 | unclassified viruses | Jingmen tick virus | + | + |
| 17 | unclassified viruses | Liman tick virus | + | + |
| 18 | unclassified viruses | Manly virus | + | + |
| 19 | unclassified viruses | Tick borne tetravirus like virus | + | + |
| 20 | *Chuviridae* | Wuhan tick virus 2 | − | + |
| 21 | *Nairoviridae* | Huangpi Tick Virus 1 | − | + |
| 22 | *Nairoviridae* | Taggert virus | − | + |
| 23 | *Phenuiviridae* | Lihan tick virus | − | + |
| 24 | *Phenuiviridae* | Rhipicephalus associated phlebovirus 1 | − | + |
| 25 | *Rhabdoviridae* | Wuhan Tick Virus 1 | − | + |
| 26 | unclassified viruses | Henan tick virus | − | + |
| 27 | unclassified viruses | Lone star tick associated virus-1 | − | + |
| 28 | unclassified viruses | Shanxi tick virus 1 | − | + |
| 29 | *Chuviridae* | Chuviridae sp. | + | − |
| 30 | *Chuviridae* | Hebei mivirus 2 | + | − |
| 31 | *Chuviridae* | Karukera tick virus | + | − |
| 32 | *Chuviridae* | Xinjiang mivirus 1 | + | − |
| 33 | *Orthomyxoviridae* | Ohshima virus | + | − |
| 34 | *Phenuiviridae* | Lone Star virus | + | − |
| 35 | *Phenuiviridae* | Okutama tick virus | + | − |
| 36 | *Rhabdoviridae* | IRE/CTVM19 associated rhabdovirus | + | − |
| 37 | *Rhabdoviridae* | Tacheng Tick Virus 3 | + | − |
| 38 | unclassified viruses | Hubei sobemo like virus 15 | + | − |
| 39 | unclassified viruses | Norway mononegavirus 1 | + | − |

**Supplementary Table S7.** The accession numbers of the virus sequences discovered in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Virus name** | **Strain** | **Segment** | **Accession number** |
| Henan tick virus | SZSX | S | OR573899 |
| M | OR573900 |
| L | OR573901 |
| Okutama tick virus | XYNZ | S | OR573902 |
| L | OR573903 |
| Dabieshan tick virus | HGMC | S | OR573904 |
| L | OR573907 |
| HGYS | S | OR573905 |
| L | OR573908 |
| XYNZ | S | OR573906 |
| L | OR573909 |
| Jingmen tick virus | HGMC | 1 | OR573910 |
| 2 | OR573912 |
| 3 | OR573914 |
| 4 | OR573916 |
| XYNZ | 1 | OR573911 |
| 2 | OR573913 |
| 3 | OR573915 |
| 4 | OR573917 |

**Supplementary figures**

**6541 supplementary-Figure-s1Supplementary Figure S1.** Phylogenetic trees were constructed for Henan tick virus based on the amino acid sequences of nucleoproteins (**A**) and glycoprotein (**B**). The red are the viruses disvovered in this study.

**6541 supplementary-Figure-s2**

**Supplementary Figure S2.** Phylogenetic trees were constructed for Dabieshan tick virus and Outama tick virus based on the amino acid sequences of nucleoproteins of genus *Uukuvirus* and *Phlebovirus* in family *Phenuiviridae* (**A**), the nucleotide sequence of DBSTV nucleoprotein (**B**), the nucleotide sequences of OKTV nucleoprotein and RdRp, respectively (**C** and **D**). The new strains discovered in this study were indicated in red.