**Supplementary Data**

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

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**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**

**Supplementary 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.

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**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.